From: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs
To: Morissette, Rachel; West-Barnette, Shayla
Subject: RE: Clarification for GRAS Notice No. GRN 000620 (GOS)
Date: Monday, May 09, 2016 6:47:15 PM
Attachments: Nestle GOS response to FDA questions May 9 2016.pdf

Dear Drs. Morissette and West-Barnette,

Attached is the Nestlé response. As indicated in our letter, we will be providing additional information on one of the questions tomorrow.

Regards,

Cheryl

Cheryl Callen
Sr. Director Regulatory Affairs
Nestle Infant Nutrition / Gerber Products Company
973-593-7494 office
201-650-1561 mobile
cheryl.callen@us.nestle.com

From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Thursday, May 05, 2016 8:23 AM
To: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs <Cheryl.Callen@us.nestle.com>
Subject: RE: Clarification for GRAS Notice No. GRN 000620 (GOS)

Hi Cheryl,
Monday is fine. I will be out of the office from May 7-17. You can cc Dr. Shayla West-Barnette (Shayla.westbarnette@fda.hhs.gov) in my absence and she will make sure that the responses are forwarded to the reviewers so there isn't a delay while I'm away. I'll inform her to look for your email. Please let us know if you have further questions.
Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Pkwy, HFS-255
College Park, MD 20740-3835
Email: Rachel.Morissette@fda.hhs.gov

From: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs [mailto:Cheryl.Callen@us.nestle.com]
Sent: Wednesday, May 04, 2016 5:04 PM

To: Morissette, Rachel
Subject: RE: Clarification for GRAS Notice No. GRN 000620 (GOS)

Hi Rachel,

We intend to submit our response on Monday, May 9th. I wanted to confirm that this is consistent with your interpretation of "within 10 working days". Your letter was dated April 25.

Thanks

Cheryl

From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Wednesday, April 27, 2016 9:29 AM
To: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs <Cheryl.Callen@us.nestle.com>
Subject: RE: Clarification for GRAS Notice No. GRN 000620 (GOS)

Thanks!

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Pkwy, HFS-255
College Park, MD 20740-3835
Email: Rachel.Morissette@fda.hhs.gov

From: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs [mailto:Cheryl.Callen@us.nestle.com]
Sent: Wednesday, April 27, 2016 9:27 AM
To: Morissette, Rachel
Subject: RE: Clarification for GRAS Notice No. GRN 000620 (GOS)

Dear Rachel ,

Thank you so much for reaching out. I did receive your email and we are working on our response. I apologize for not responding sooner.

Regards,

Cheryl

Cheryl Callen

Sr. Director Regulatory Affairs
Nestle Infant Nutrition / Gerber Products Company
973-593-7494 office
201-650-1561 mobile
cheryl.callen@us.nestle.com

From: Morissette, Rachel [mailto:Rachel.Morissette@fda.hhs.gov]
Sent: Wednesday, April 27, 2016 9:26 AM
To: Callen,Cheryl,FLORHAM PARK,Regulatory Affairs <Cheryl.Callen@us.nestle.com>
Subject: Clarification for GRAS Notice No. GRN 000620 (GOS)

Dear Ms. Callen,
Due to the time-sensitive nature of the request, I wanted to make sure that you received the email that I sent a few days ago regarding clarification of your notice GRN 620. Please let me know if you have further questions.
Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Pkwy, HFS-255
College Park, MD 20740-3835
Email: Rachel.Morissette@fda.hhs.gov

From: Morissette, Rachel
Sent: Monday, April 25, 2016 8:52 AM
To: 'cheryl.callen@us.nestle.com'
Subject: Clarification for GRAS Notice No. GRN 000620 (GOS)

Dear Ms. Callen,
Please find attached a letter requesting clarification on several issues with regard to GRN 000620 on galacto-oligosaccharides (GOS).

Best regards,

Rachel

Rachel Morissette, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Pkwy, HFS-255
College Park, MD 20740-3835

Email: Rachel.Morissette@fda.hhs.gov

Nestlé Nutrition U.S.
12 Vreeland Road • Box 697
Florham Park, New Jersey 07932-0697

Nestlé | Nutrition

May 09, 2016

Rachel Morissette, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Phone: 240-402-1212
Email: Rachel.Morissette@fda.hhs.gov

Dear Dr. Morissette:

Please see the following responses to the agency's questions on GRAS Notice No. 000620 for the use of galacto-oligosaccharides in non-exempt term infant formula that was submitted by Nestlé Nutrition on December 22, 2015.

FDA (Q1): There are no heavy metal specifications listed in the notice. At a minimum, specifications should be listed for arsenic, lead, cadmium, and mercury showing batch analyses from at least three non-consecutive lots.

.

We have updated the specifications on the heavy metals with the following maximum limits for the Nestlé GOS ingredient and provide the additional measured analytical results on batches 341778, 347230, 291604 and analytical results from batch 287060 (this batch has not been part of the original Table II.C-2, p. 9):

	Nestlé GOS specification [mg/kg]	Analytical results lot 291604 [mg/kg]	Analytical results lot 341778 [mg/kg]	Analytical results lot 347230 [mg/kg]	Analytical results lot 287060** [mg/kg]
Pb	≤ 0,02	Nd (<0.0046)*	Nd(<0.0046)*	Nd (<0.0046)*	Nd (<0.0046)
As	≤ 0,02	Nd (<0.009)	***	Nd (<0.009)	Nd (<0.0084)
Cd	≤ 0,02	Nd (<0.0023)	***	Nd (<0.0023)	Nd (<0.0023)
Hg	≤ 0,02	Nd (<0.007)	Nd (<0.007)	Nd (<0.007)	Nd (<0.007)

*result has already been stated in originally submitted GRAS notification (Table II.C-2, p. 9)
** analytical results of lot 287060 have not been part of the originally submitted GRAS notification (Table II.2-C, p. 9) they are included here to provide results on 3 non-consecutive lots.
*** As and Cd were not analyzed for this lot 341778. Therefore analytical results for all 4 metals are included for a 4th sample, lot 287060.

FDA (Q2): The notice states that a specification for Cronobacter sakazakii (Cronobacter spp) is not necessary in light of the fact that Nestlé GOS is added to infant formula prior to thermal processing. However, Cronobacter spp can form biofilms that render cleaning and sanitizing techniques less effective, potentially introducing contamination. Therefore, FDA requests that Nestlé provide an appropriate specified limit for Cronobacter spp and batch analyses from three non-consecutive lots.

Nestlé (A2): As discussed in the notice, Nestlé GOS is manufactured exclusively for Nestlé infant formula applications manufactured using wet blending technique and is always added to the formula prior to heat treatment of the wet blend. Accordingly, like other ingredients used in Nestlé's wet blending technique and added before heat treatment, Nestle GOS is not routinely tested for *Cronobacter spp.* All infant formula to which Nestle GOS has been added would be compliant with the microbial requirements for such products.

Nestle will be providing additional information regarding the processes in place at the formula manufacturing facility in a future communication.

FDA (Q3): A specified minimum content is given for sialyllactoses that originate from the demineralized whey permeate. Please provide the typical range of concentration or if there is a specified upper limit for these compounds in Nestlé GOS.

Nestlé (A3): Siallyllactose is naturally present in cow's milk and part of the oligosaccharides. In the Nestle GOS ingredient, siallyllactose is typically present in a concentration between 0.2 and 0.3 g/100g (refer to table II C-2 p 9 in the Notification). An upper limit is not specified because the concentrations are fairly consistent within a narrow range.

FDA (Q4) Table II.C-2 lists citric acid as an analyte, although no specified limit is given. Please describe the source of citric acid in Nestlé GOS and whether a specified limit is established.

Nestle (A4): Citrate (measured as citric acid content) is naturally present in cow's milk in concentrations of 0.139 to 0.147% (Yusa *et al*., 1969[1]), Most of this citrate is transferred to the whey fraction during cheese manufacturing and therefore it is also present in the whey permeate. Since Nestlé GOS is derived from whey permeate, it still contains the citrate from the milk. Citrate is not added to the Nestlé GOS ingredient. Although the content of citrate in Nestlé GOS will vary slightly due to natural variation, it does not require critical control limits and no specification has been proposed.

FDA (Q5) On pages 15 and 22, the notice states that a maximum use level of 8 g GOS/L is permitted by FSANZ and the Ministry of Health of the PRC. Furthermore, a reference from the Ministry of Health of the PRC is cited as an unofficial-English translation, but FDA is unable to locate this reference. Please provide a certified-English translation of this reference. If the assessment by the Ministry of Health of the PRC is used to establish "general recognition" of safety, please clarify how a publication or declaration that is not readily available in English can be used to establish general recognition of the safety of this new use level of GOS.

Nestlé (A5): The citation to the 8g GOS/L use level for infant formula GB 2760-2011 is incorrect. The correct reference is from GB 14880-2012 <Food Safety National Standard, Standard for Use of Nutrition Fortifier>. According to GB 14880-2012, <Food Safety National Standard, Standard for Use of Nutrition Fortifier>, GOS is permitted for formulas for infant and young children and cereal-based complementary foods for infant and young children, singularly or combined, total content of such category not exceed 64.5g/kg, which is equal to maximum use level of 8 g GOS/L in ready-to-eat reconstituted formula. A copy of GB 14880-2012 is included and translation of the table relevant to GOS use is provided below.

[1] Japanese Journal Zootechnical Science 1969 Vol. 40 pp. 32-36

表 C.2 仅允许用于部分特殊膳食用食品的其他营养成分及使用量			
营养强化剂	**食品分**类号	**食品**类别（名称）	**使用量**[a]
低聚半乳糖（乳糖来源）	13.01 13.02.01	婴幼儿配方食品 婴幼儿谷类辅助食品	单独或混合使用，该类 **物**质 总量不超过 64.5 g/kg
低聚果糖（菊苣来源）			
多聚果糖（菊苣来源）			
棉子糖（甜菜来源）			
a **使用量**仅限于粉状产品，在液态产品中使用需按相应的稀释倍数折算			

Table C.2 Nutrients Allowed For Special Dietary Uses			
Nutrition Supplements	Food Category	Food Category (Name)	Usage[a]
GOS (Source Lactose)	13.01	Formula Food For Infant and Young Child	Singly or in combination
FOS (Source Chicory)			
Polyfructose (Source Chicory)	13.02.01	Supplementary Foods (Infant Cereals)	Not to exceed 64.5 g/Kg
[a]For use only in powdered product, liquid products use level must be adjusted according to appropriate dilution factor			

Nestlé does consider the assessment by the Ministry of Health of the PRC to be applicable to the GRAS status of GOS for use in infant formula. Although GB 14880-2012 is written in Chinese, advances in information technology allow for rapid and user-friendly online translation of Chinese script into any language. For example, the above table was translated using free translation services hosted by Google[2] and Microsoft[3]. Based upon the general availability of GB 14880-2012[4] and availability of free online software that can be accessed by scientific experts or the general public, the regulatory status of GOS for use in term infant and follow-on formula as described in GB 14880-2012 can be considered applicable to the GRAS status of the ingredient.

FDA (Q6): In the latest recommendation from EFSA [EFSA Journal 12: 3760 (2014)], the Panel on Dietetic Products, Nutrition, and Allergies states:
"The Panel considers that there is no evidence to change the previous conclusions of the SCF (2001d) that a mixture of 90% GOS and 10% of high-molecular-weight FOS is safe under the current conditions of use (i.e. ≤0.8 g/100 mL) in IF and FOF. The safety of any other non-digestible oligosaccharides or any new mixture of non-digestible oligosaccharides in IF and FOF should be established by clinical evaluation."

Based on this statement, it would appear that EFSA, unlike FSANZ and the Ministry of Health of the PRC, has not yet concluded that GOS used at 8 g/L is safe. Please discuss EFSA's position with respect to the general recognition that this new use level is safe.

Nestlé (A6): In accordance with EFSA's view that new mixtures of non-digestible oligosaccharides be evaluated for safety using clinical evaluation, Nestle notes that Nestle GOS has been clinically evaluated in 3 trials at a use level of 8 g/L (Simeoni *et al*., 2016[5]; Hascoet *et al*., 2016; Cooper *et al*., 2015). These studies were not available to EFSA during the aforementioned evaluation in 2014. All of these studies are now in the public domain as a published peer-reviewed article (Simeoni *et al.,* 2016) or as public abstracts that have been presented at International congresses (PAS & ESPGHAN). All three studies are described within Nestlé's notice (IV.B.3.1Studies of Nestlé GOS in Infants), however, the work by Simeoni *et al*., and Hascoet *et al*., were not yet available in the public domain.

The study by Simeoni *et al*. (2016) was a double-blind placebo controlled trial conducted in 115 healthy term infants, aged ≤14 days, randomized to one of two formula fed groups: 1) a test starter IF with Nestlé GOS (8 g/L in reconstituted formula) + *B.lactis* ($1x10^7$ CFU/g of powder formula) (n=39) or 2) a control IF (n=37) for a 12-week feeding period. Infants from mothers who elected to breastfeed were included as a breastfed reference control group (n=39). The authors reported that their "…*study was powered to demonstrate non-inferiority*

[2] https://translate.google.com/
[3] https://www.bing.com/translator
[4] http://www.cirs-group.com/food/regulations&standards/GB_14880-2012_usage_nutrition_enrichment.html
[5] Environmental Microbiology (2016) doi:10.1111/1462-2920.13144

for the test formula compared with the control formula for growth." and "*All anthropometric parameters (weight gain, length and head circumference) did not differ between groups from inclusion until 3 months of age*." In addition consumption of the test formula containing Nestlé GOS was well-tolerated by all infants and the test and control formula groups showed no differences in "spitting up", vomiting, crying, colic, flatulence, or irritability.

The study by Hascoet et al., (2016) was a large multi-center double-blind placebo controlled trial conducted in 413 healthy term infants, randomized to one of two groups formula fed groups: 1) a test starter IF with Nestlé GOS (8 g/L) + *B. lactis (*10^7 cfu/g of powder) (n= 206) or 2) a control IF (n=207) from birth up to 6 months of age. A breastfeeding group (n=63) was included as a reference. All anthropometric parameters of infants consuming either test or control formula were not significantly different, and aligned with WHO growth standards. Between test and control groups there was no significant difference in frequency of vomiting, spitting up, crying, restlessness, and incidence of colic. This study demonstrated non-inferior growth in infants randomized to receive Nestlé GOS relative to control infants and incidences of adverse events including infectious episodes were not different between the groups.

The study by Cooper *et al*., (2015) was another large (n=421) multi-center double-blind controlled trial. Infants were randomized to one of 4 parallel groups based on delivery method. The first two groups consisted of caesarean-delivered infants provided Nestlé GOS (8 g/L) + *B. Lactis* (n=92) or a control formula (n=101); the second two groups consisted of vaginally delivered infants randomized the same Nestle GOS + *B. Lactis* (n=115) or control (n=113) formulas. The primary outcome included comparisons of mean weight gains between 10 days and 4 months of age, the secondary outcomes were length, head circumference and body composition measurements up to 12 mo of age. Digestive tolerance was assessed and morbidity was evaluated by frequency of adverse events. No statistically significant differences between the Nestlé GOS and control groups, regardless of delivery mode, were reported with respect to weight, length and HC. No significant differences between groups were observed for all other anthropometric indices. No increased incidence of adverse events was observed between the Nestlé GOS and the control groups during the study period.

All 3 studies were prospective placebo controlled double-blind studies conducted in healthy term infants who consumed infant formula supplemented with Nestlé GOS at 8 g/L from birth through ≥3 months of age. The Studies by Cooper *et al*., (2015) and Hascoet *et al*., (2016) were 12 month intervention studies in large groups of infants and therefore were particularly sensitive studies for obtaining information on tolerance, adverse effects, and measures of growth in accordance with the requirements of U.S. FDA and the American Academy of Pediatrics (AAP) (AAP, 1988[6]).

FDA (Q7) In Williams et al. (2014), the authors state:

"Infants fed EF8 [GOS supplemented formula at 8 g/L] were, however, the most likely to have watery stools during most study periods, and they had a significantly higher percentage of watery stools than infants fed CF [no GOS] during the initial study period. These data indicate that 4 g GOS/L is preferable to 8 g GOS/L for prebiotic supplementation of infant formula."

In addition, they report:

"[t]here was a significantly higher proportion of serious adverse events, primarily respiratory tract infections, in the EF8 versus the EF4 [GOS supplemented formula at 4 g/L] and CF groups (P<0.05)."
Please comment on these statements, which appear to contradict the general recognition that GOS at a use level up to 8 g/L is safe.

[6] AAP (1988). *Clinical Testing of Infant Formulas With Respect to Nutritional Suitability for Term Infants*. (Prepared under FDA contract 223-86-2117). Prepared by Elk Grove Village (IL): American Academy of Pediatrics (AAP) for College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Nutritional Products, Labeling, and Dietary Supplements. Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/InfantFormula/ucm170649.htm

Nestlé (A7): The study by Williams *et al*., (2014) was included as part of the comprehensive review of the literature of all infant and children studies conducted with GOS from January 2014 through December 2015 and was presented in a summary table. This study was not conducted with Nestlé GOS. Nestlé reviewed this study in detail during the company's GRAS determination and agrees that further discussion of the authors' findings and significance of these findings to the GRAS determination is warranted.

Stool consistency and frequency data was collected throughout the approximate 119 day study. Infants fed EF8 [GOS g/L] had a significantly higher percentage of watery stools relative to control from study day 1 to day of life (DOL) 14, which was not reported at other time point during the study. Stool frequency did not differ between the groups. At DOL 14 infants were examined by a physician with special attention paid to signs of dehydration. In addition, urine specific gravity was also measured at this time point. There was no clinical indication of dehydration and urine specific gravity was within normal limits, which suggests the higher percentage of watery stools was not contributing to any safety concerns. . There was no clinical indication of dehydration and urine specific gravity was within normal limits, which supports a conclusion that the higher percentage of watery stools does not reflect a safety signal, therefore does not change prior general recognition of safety.

The observation of a higher proportion respiratory tract infections in the EF8 versus the EF4 and CF groups in this study was not seen in any of the other clinical trials conducted with GOS since Jan 2014 (as reviewed in the Nestle GOS notification. Most relevant to the general recognition of safety of this GOS ingredient was the lack of respiratory related adverse events in the 3 clinical trials using Nestle GOS at 8 g/L.

Notably, Cooper *et al*., (2015) and Hascoet *et al*., (2016) evaluated the long-term consumption (6 months) of GOS (Nestlé GOS) at a use level of 8 g/L and follow-up till 12 months. These were large multi-center placebo controlled studies and with a higher powered study design and longer-term follow-up would be more sensitive to detect potential adverse effects of GOS on incidences of adverse effects/disorders and no significant differences in respiratory tract infections/disorders were observed in the infants randomized to the GOS groups relative to controls (See Tables below for summary of adverse events).

Number of infants (%) with specific adverse events (Hascoet *et al*., 2016)

SOC	Test n=179	Control n=180	Breastfed n=59
Respiratory, thoracic, and mediastinal disorders	17 (9.4)	12 (6.7)	2 (3.4)
Gastrointestinal disorders	74 (41.1)	84 (46.9)	31 (52.5)
Skin and subcutaneous tissue disorders	24 (13.3)	25 (14.0)	12 (20.3)

SOC, system organ class according to World Health organization adverse drug reaction terminology

Adverse Events (Cooper *et al*., 2015)

Adverse event Category	Delivery Mode					
	Vaginal			Caesarean		
	Test (N=115)	Control (N=113)	p*	Test (N=92)	Control (N=101)	p*
All respiratory infections/symptoms	57 (49.6%)	51 (45.1%)	0.511	50 (54.3%)	46 (45.5%)	0.250
Diarrhea	15 (13.0%)	18 (15.9%)	0.576	15 (16.3%)	11 (10.9%)	0.297
Gastrointestinal conditions	21 (18.3%)	28 (24.8%)	0.261	18 (19.6%)	19 (18.8%)	1.000
Skin disorders	32 (27.8%)	33 (29.2%)	0.884	27 (29.3%)	22 (21.8%)	0.250

Data are number infants with at least one event (%). N=number of infants; %=percentage. * Exact Fisher test p value.

FDA (Q8): Underwood et al. (2014) observed that two infants in the GOS study group "developed blood-streaked stools with mild abdominal distention." The authors hypothesized that the observation "may be

coincidental or may be related to the osmolality or composition of the [GOS] syrup itself." Please comment on why this observation is not a cause for safety concern.

Nestlé (A8): The study by Underwood *et al*., (2014) was identified during Nestlé's updated literature search and was evaluated during the GRAS determination process consistent with the requirement to consider all generally available data and information, both positive and negative, which may be pertinent to the safety of the intended uses of the substance in food. As Nestle is seeking GRAS determination of Nestlé GOS powder in healthy term infants at 7.8g/L, we did not expand on this in our initial submission Nestlé noted that the study by Underwood *et al*., (2014) was conducted in a very small sample size (n=6) of premature infants. Nestlé notes that a time-line for the development of blood-streaked stools with mild distension was not reported, which complicates interpretation of the dose-response relationship as the study was a dose-escalation study with GOS doses increasing from 2.5 g/L on week 1 to large concentrations of 20 g/L on week 5 (See Table below). By week three of the study the concentration of GOS in the test formula far exceeds levels proposed for Nestle GOS as described in GRN 620.

Dosage Schedule (Underwood *et al*., 2014)					
Prebiotic	Week 1	Week 2	Week 3	Week 4	Week 5
GOS (g/L of formula	2.5	5	10	15	20

Given the absence of similar findings in any of the studies in healthy term infants, little relevance can be attached to Nestlé's GRAS determination of Nestlé GOS powder in healthy term infants at 7.8g/L.
Nestlé is not aware of other reports of "blood streaked stools" associated with GOS consumption at use levels of 8 g/L, and not seen in our clinical studies (Simeoni *et al*., 2016; Hascoet *et al*., 2016; Cooper *et al*., 2015).

FDA (Q9): In Meli et al. (2014), the authors state:
"Further studies are needed to fully explore the digestive tolerance of BMOS [GOS] formula. The addition of BMOS to infant formula resulted in more frequent, less hard stools compared with control formula; however, a higher incidence of colic was also detected."
The authors also indicate that:
"[a]dditional studies are planned with lower levels of BMOS," suggesting that these investigators believe that the observed adverse effect may be significant.
Please comment on how their observations are consistent with the general recognition that 7.8 g/L, as opposed to the accepted 7.2 g/L, is safe.

Nestlé (A9): This study which evaluated 10 g/L GOS was critically reviewed in GRN 620 on page 23 of the notice, including its relevance to the GRAS determination. Key outcomes of this trial include adequate power to asses safety measures of growth with no differences reported between groups. There were also no statistically significant differences in adverse events or clinical safety indices. While there were no differences in caregivers' reports of tolerance including episodes of colic as noted above, however, there were differences in investigator-diagnosed colic. Nestlé discussed in detail the unexplained discrepancy between the caregiver reported incidences of colic and the investigator reported incidences of colic. In the Notice Nestle reported the following:

"*Caregiver-reported colic was not significantly different between infants administered infant formula with prebiotic (Nestlé GOS) versus control; however, caregivers were asked to record episodes of colic (defined as bouts of intense, inconsolable crying with painful facial expressions and pulling up of the legs) for only 3 days prior to each visit at 14 days and at ages 1, 2, 3, 4, 6 and 12 months. The 3-day collection period may not have been sufficient to identify episodes of colic. The investigator-reported definition of colic required the occurrence of colic for 3 or more hours per day and for at least 3 days per week for at least 1 week. It is not clear how this information could have been collected by the investigator, given that it was not collected as such in the caregiver diaries. Nestlé also notes that the investigator definition of colic used by the authors would not be defined as colic under the Wessel Criteria whereby colic is defined as unexplained paroxysmal bouts of fussing and crying that lasted for >3 hours a day, for >3 days a week, for >3 weeks (Garrison and Christakis, 2000).*"

Due to these limitations in study design, Nestle notes that no conclusion on an association between treatment and investigator reported colic could be established.

Nestlé's opinion was also supported by critical evaluation by an independent Expert Panel who stated the following on page 11 of the Expert Panel report:

"*… The Expert Panel considered the authors conclusions on tolerance (i.e., colic) to be complicated by limitations in the study design and placed greater emphasis on the care-giver records over investigator reported outcomes…*"

In reference to the authors' comment about additional studies are planned at lower levels, 3 separate and independent trials evaluating the administration of Nestlé GOS at a concentration of 8 g/L have been published in the peer-reviewed literature as full manuscripts or as preliminary results in abstracts (Simeoni *et al*., 2016; Hascoet *et al*., 2016; Cooper *et al*., 2015). For example, Simeoni *et al*., reported that consumption of infant formula supplemented with Nestlé GOS at a use level of 8 g/L for 3 months did not result in differences in "… *'spitting up', vomiting, crying, colic, flatulence and irritability*…" relative to control infants receiving standard infant formula. In the study by Hascoet *et al*., (2016) the consumption of infant formula containing Nestle GOS at 8 g/L for 6 months did not result in significant differences "… *in frequency of vomiting, spitting up, crying, restlessness, and incidence of colic between Test and Control groups.*" The study by Cooper *et al*., (2015), another long-term study evaluating the administration of infant formula containing Nestlé GOS to healthy term infants, reported that there were no statistically significant differences between infants receiving the test formula containing Nestlé GOS compared to the control formula regarding the percentage of days with unusual stool odor, flatulence, spitting up, vomiting, crying, fussing, or colic, regardless of mode of delivery. These studies demonstrate that the use of Nestlé GOS at 8 g/L did not result in statistically significant increases in colic or other adverse effects relative to control infants administered formula that is absent Nestlé GOS thus supporting the general recognition that 7.8 g/L of Nestle GOS is safe.

FDA (Q10): With respect to its use in infant formula, it is generally recognized that GOS is added along with FOS at a 9:1 ratio, not exceeding a total amount of 8 g/L GOS+FOS. Please briefly discuss the significance, or lack thereof, of a 9:1 (GOS: FOS) ratio in IF use. Also, please discuss Nestlé's conclusion that deviating from the standard formulation is not generally recognized to be a safety issue.

Nestlé (A10): Galacto-oligosaccharides (GOS), and fructo-oligosaccharides (FOS) are β-linked linear oligomers that resist hydrolysis by salivary and small intestinal digestive enzymes. These oligomers are not absorbed and are therefore transported intact to the large intestine where they are fermented by the indigenous microbiota. At their basic molecular structure, GOS and FOS are comprised of simple monosaccharides (glucose, galactose, and fructose) that are common to the diet. During microbial fermentation these monosaccharides are expected to be metabolized by the resident anaerobic microbiota *via* the Embden-Meyerhof-Parnas pathway resulting in the production of short chain fatty acids, CO_2 and H_2 gas (common and innocuous dietary metabolites) (Miller and Wolin, 1996; Suarez et al., 1999; Smiricky-Tjardes et al., 2003). It is these products of microbial fermentation, short-chain fatty acids in particular, which result in the reduced pH, osmotic effects, and improvements in stool consistency that are observed in infants consuming human milk. As noted by the FDA, GOS is GRAS for use in infant formula at a use level of up to 7.2 g/L with or without 0.8 g of added FOS/Inulin (GRN 569, 495, 334, 286). Since FOS and GOS are fermented to similar metabolites by the gut microbiota, both compounds will have similar physiological effects in the large intestine. Provision of GOS at a use level of 7.8 g/L without the addition of 0.8 g/L of FOS is a quantitatively small change in the relative amounts of GOS and FOS. Accordingly, based on their similar metabolic profile it is reasonable to expect no adverse physiological consequences provided that the total amount of GOS/FOS oligomers do not exceed 8 g/L. This conclusion is corroborated by available clinical studies conducted in infants using Nestle GOS at a use level of 8 g GOS/L (Cooper *et al*., 2015; Hascoet *et al*., 2016; Simeoni *et al*., 2016), as well as by a similar conclusion by the Food Standards Agency Australia New Zealand (FSANZ), who stated "*based on the available evidence, FSANZ concludes that infant and follow-on formula containing up to 8 g/L of inulin derived substances and/or*

GOS, singularly or combined, in any ratio, are unlikely to pose a risk to infants" (FSANZ, 2008) [7,8].

FDA (Q11): On page 15, the notice states:

"In addition, infants consuming human milk are exposed to oligosaccharides at levels higher than 7.8 g/L. For example, human milk oligosaccharide concentrations of 25 and 12 g/L have been reported in human colostrum and mature milk samples, respectively (Kunz et al. 1999, 2000)."

Given that exposure calculations are based on the use level AND the amount consumed, please provide a statement or comment that relates the total amount of oligosaccharide in breast milk consumed to the total amount of GOS from the intended use level in infant formula that the infants are expected to consume.

In addition, Kunz et al. (1999) state that:

"[v]arious actions of fucosyltransferases and sialyltransferases led to the identification of more than a 100 different oligosaccharide structures in human milk that have been characterized so far".

Therefore, it is not clear how much of the oligosaccharides identified in human colostrum and mature milk samples are actually GOS-like. Since the subject of the notice is specific to GOS, please clarify the above statement regarding how much of the total oligosaccharides in human colostrum/mature milk is actually structurally similar to Nestlé's GOS.

Nestlé (A11): Nestle recognizes that human milk oligosaccharides are structurally and compositionally different from GOS and that there are only trace amounts of GOS in human milk (Newburg *et al*., 2015[9]). The purpose of this sentence was not to make an exact comparison of Nestle GOS to GOS in human milk, but to compare the non-digestible Nestle GOS to the non-digestible human milk oligosaccharides in terms of osmotic and fermentable load provided to a term newborn infant. It was to also emphasize that human milk contains large amounts of non-digestible oligosaccharides provided to breastfed infants naturally at levels of 5-15 g/L or 4 to 12 g per day (EFSA (2014)).Since it is not feasible to add a similar oligosaccharide composition to infant formula (SCF 2003), the addition of oligosaccharides like GOS while not precisely duplicating the structure of those found in human milk, may mimic at least some of their metabolic and functional effects.

Thank you for the opportunity to provide this additional information. If you have any further questions, please let us know.

Sincerely,

Cheryl Callen
Sr. Director Regulatory Affairs

---

[7]FSANZ. 2008. Addition of Inulin/FOS & GOS to Food: Final Assessment Report: Proposal P306. Food Standards Australia New Zealand (FSANZ); Canberra, Australia. Available from: http://www.foodstandards.gov.au/_srcfiles/P306%20FOS%20&%20GOS%20FAR%20FINALv2.pdf.

[8]Professor John Cummings (Emeritus Professor of Experimental Gastroenterology, University of Dundee, Scotland) and Professor Glenn Gibson, Head of Food Microbial Sciences, University of Reading, England.

[9] J Nutr. 2016 Feb;146(2):358-67