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November 7, 2011

Antonia Mattia, Ph.D., Director
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Toni:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Nestle Nutrition U.S., through me as its agent, hereby provides notice of a claim that the use of *L. reuteri* strain DSM 17938 in infant formula as described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because Nestle Nutrition has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification are provided. Each copy includes the summary conclusion of the GRAS expert panel as well as the signatures of the seven panel members.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Generally Recognized as Safe (GRAS) Determination of *Lactobacillus reuteri* Strain DSM 17938 for Addition to Term Infant Formula

Prepared for
Nestle Nutrition
Florham Park NJ

Prepared by
JHeimbach LLC
Port Royal VA

November 2011

1. GRAS Exemption Claim

Nestle Nutrition U.S., through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula intended for term infants as described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Nestle Nutrition has determined through scientific procedures that this use is generally recognized as safe (GRAS).

(b) (6)

November 7, 2011
Date

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

1.1. Name and Address of Notifier

Nestle Nutrition U.S.
12 Vreeland Road
Florham Park NJ 07932

Contact: Brian D. Kineman, Ph.D., Manager, U.S. Regulatory Affairs
Telephone: 973-593-7607
Facsimile: 973-593-7664
E-mail: brian.kineman@us.nestle.com

1.2. Name of GRAS Organism

The subject of this GRAS determination is the probiotic bacterium *Lactobacillus reuteri* strain DSM 17938. This strain was the subject of GRAS Notice No. GRN 000254, submitted on May 9, 2008. The FDA response letter was dated November 18, 2008. As is discussed further below, the entirety of GRN 254 (with the exception of the section on intended use) is incorporated by reference in the present GRAS notice.

1.3. Intended Use and Consumer Exposure

L. reuteri strain DSM 17938 is intended to be added to routine powdered whey-based infant formula intended for term infants to achieve a minimum level of 10^6 colony-forming units (cfu) per gram powder. The addition level to assure the presence of 10^6 cfu/g will be consistent with current good manufacturing practice (cGMP), but in no case higher than 10^8 cfu/g. With normal dilution of the powdered formula in water at 13.5 g/100 ml, the concentration of *L. reuteri* in the prepared formula would be 1.35×10^7 cfu/100 ml, producing an estimated intake of 10^8 cfu/day.

1.4. Basis for GRAS Determination

Nestle Nutrition's GRAS determination for the intended use of *L. reuteri* strain DSM 17938 is based on scientific procedures as described under 21 CFR §170.30(b).

Determination of the safety and GRAS status of the intended addition to infant formula of *L. reuteri* strain DSM 17938 was made through the deliberations of an Expert Panel consisting of individuals qualified by scientific training and experience to evaluate the safety of infant formula

and ingredients including probiotic microorganisms. The Panel included the individuals who determined the GRAS status of the addition of the strain to conventional foods (Berthold Koletzko, M.D., Daniel O'Sullivan , Ph.D., Mary Ellen Sanders, Ph.D., and John Thomas, Ph.D.) along with three additional panelists intended to bring additional expertise in pediatric gastroenterology and intestinal function to the panel, William Klish, M.D., Walter Mihatsch, M.D., and Kelly Tappenden, Ph.D. The Panel critically reviewed and evaluated the generally available information and the potential exposure of infants to *L. reuteri* DSM 17938 resulting from its intended use, and individually and collectively concluded that no evidence exists in the available information on *L. reuteri* strain DSM 17938, its parent strain ATCC 55730, other *L. reuteri* strains, or other probiotic bacteria, that demonstrates or suggests reasonable grounds to suspect a hazard to infants under the intended conditions of use of *L. reuteri* strain DSM 17938.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and related information would reach the same conclusion. Therefore, addition of *Lactobacillus reuteri* strain DSM 17938 to infant formula under the conditions described is GRAS by scientific procedures.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHeimbach LLC, 923 Water Street, P.O. Box 66, Port Royal, Virginia 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

2. Identity of the Organism

Extensive information regarding the identification, characterization, and production of the strain is contained in GRN 254, which is incorporated by reference.

One change is that the description of the curing of two plasmids that harbored antibiotic resistance genes and the evidence for the substantial equivalence of the parent *L. reuteri* strain ATCC 55730 and daughter strain DSM 17938 was cited in GRN 254 as an unpublished report to BioGaia. This work has since been published as Rosander et al. 2008, and the full citation is provided in the reference section.

3. Intended Use and Exposure

L. reuteri strain DSM 17938 is intended to be added as a probiotic microorganism to routine powdered whey-based infant formula intended for term infants to achieve a minimum level of 10^6 cfu/g. The addition level to assure the presence of 10^6 cfu/g will be consistent with cGMP, but will not exceed10^8 cfu/g. With normal dilution of the powdered formula in water at 13.5 g/100 ml, the concentration of *L. reuteri* in the prepared formula would be 1.35 x 10^7 cfu/ 100 ml.

The target ingestion of the strain is 10^8 cfu/day, which will be achieved by an average formula intake of about 800 ml/day. According to tables of daily energy intake by formula-fed infants provided by Fomon (1993), the subpopulation of infants with the highest intake per kg body weight is boys age 14–27 days. The mean energy intake by this group is 121.1 kcal/kg bw/day and the 90th percentile is 141.3 kcal/kg bw/day. Among girls, the highest energy intake is found in the same age group, 14–27 days, and is nearly as high as boys: the mean and 90th energy intake percentiles are 117.8 and 138.9 kcal/kg bw/day. Most term infant formulas contain 67.6 kcal/100 ml when ready to consume. Therefore, to obtain 141.3 kcal energy/kg bw, an infant boy must consume 209.0 ml formula/kg bw. To reach her 90th percentile of energy consumption, 138.9 kcal/kg bw/day, an infant girl must consume 205.5 ml formula/kg bw/day. The 90th percentile of formula intake for the two sexes combined is about 207 ml/kg/day. This would result in a 90th percentile exposure of 2.7 x 10^7 cfu *L. reuteri* /kg bw/day.

Under contract with Nestec Ltd., the Laboratory of Clinical Chemistry at the University of Lausanne assessed the potential production of lactic acid enantiomers after reconstitution of 3 different powdered infant formulas and incubation at 3 different temperatures (4, 25, and 37°C) for 3 different durations (0, 2, and 6 hours). The 3 tested formulas included a control whey-based formula containing no probiotic bacteria, the same formula with 10^6 cfu *B. lactis*/g, and the same formula with 10^6 cfu *L. reuteri* strain DSM 17938/g.

The formulas were reconstituted in water at 4°C and then adjusted to the desired temperature. Ten samples were tested for concentrations of D- and L-lactate under each condition. There were no differences in the concentrations of either enantiomer among the 3 formulas at any tested temperature over any tested duration, and none of the formulas showed changes in either D- or L-lactate over time at any tested temperature. The study report concluded that "these results clearly indicate that when stated [sic] under normal use conditions or at prolonged storage up to 6 h either at 4°C, 25°C or 37°C, the reconstituted 3 formulae are stable and do not show increases of their L- and D-lactic acid concentrations."

4. Safety

This section of GRN 254, which includes the results of a genomic analysis of *L. reuteri* strain DSM 17938; *in vitro*, animal, and human studies of the strain or its parent strain (including nine studies in term infants and three in preterm infants); review articles regarding the safety of *L. reuteri*; and a discussion of the assignment of qualified presumption of safety (QPS) status to *L. reuteri* by the European Food Safety Authority (EFSA), is incorporated by reference.

Relevant research completed since preparation of GRN 254 is discussed below. It is also to be noted that EFSA has issued annual updates of QPS status in 2008, 2009, and 2010, and the status of *L. reuteri* has not been questioned.

The urinary D-lactate excretion of infants ingesting *L. johnsonii* strain La1 added to infant formula was evaluated by Haschke-Becher et al. (2008). Like *L. reuteri, L. johnsonii* produces both the D- and L-isomers of lactic acid. A total of 71 healthy infants with gestational ages of 36-44 weeks and birth weights >2500 g was enrolled; the average age of the infants was about 106 days. Twenty-six infants were breast fed, and the remaining infants were randomly assigned to receive formula containing 0 (n = 26) or 10^8 cfu probiotic/g powder (n = 19). Parents were instructed to provide 3-4 200-ml formula feedings/day to achieve daily intakes of 0.8-1.1×10^{10} cfu *L. johnsonii*. Morning urine samples were taken at baseline and after 4 weeks and analyzed for D- and L-lactate as well as creatinine; lactate excretion was expressed per mol creatinine.

Thirteen infants were withdrawn from the study, none for reasons attributed to the feeding. There were no differences in formula intake between the 2 formula groups nor among the 3 groups in growth. There were no differences in urinary D-lactate concentrations among the 3 groups at baseline, but after 4 weeks D-lactate excretion increased significantly in both formula groups as compared to the breastfed group, but the 2 formula groups did not differ in D-lactate excretion. There were no differences among the groups in L-lactate excretion at any time. The authors concluded that "current evidence does not point at any risk of lactate acidosis in healthy infants fed a formula supplemented with the probiotic strain La1."

Indrio et al. (2009) studied the effects of supplementation of infant formula with either a galactooligosaccharide-fructooligosaccharide (GOS-FOS) blend or *L. reuteri* strain DSM 17938 on gastrointestinal motility in preterm neonates. Forty-nine healthy preterm infants were enrolled in a randomized, double-blind, placebo-controlled study during their first week of life; 17 were exclusively breastfed as a human-milk reference group while 32 were randomly assigned to receive formula supplemented with 800 mg GOS-FOS/100 ml (n = 10), *L. reuteri* DSM 17938 in an oil formulation providing 10^8 cfu/day (n = 10), or placebo (n = 12) for 30 days. Anthropometric measures (body weight, length, and head circumference) were taken at enrollment and throughout the study, parents completed 24-hour tolerance recalls, and adverse events were monitored. Gastric electrical activity (EGG) recordings and ultrasound assessments of gastric emptying were made at enrollment and at the end of 30 days.

There were no differences between groups in growth or in reported adverse events. The breastfed group and infants receiving either the pre- or probiotic-supplemented formulas had significantly better EGG recordings and faster gastric emptying compared to the placebo group at the end of the 30-day intervention period. The authors concluded that, "Feeding preterm infants with a formula supplemented with prebiotics or probiotics may stimulate gastric emptying and improve maturation of the EGG activity mimicking the effect of breast milk."

In a randomized, double-blind, placebo-controlled study of infants with functional chronic constipation, Coccorullo et al. (2010) supplemented the feeding of infants with either *L. reuteri* strain DSM 17938 or placebo for 8 weeks. Forty-four infants aged at least 6 months (mean age = 8.2 months) admitted to the Gastrointestinal Endoscopy and Motility Unit with chronic constipation were randomly assigned to receive a single daily dose of 10^8 cfu L. reuteri in 5 drops of a commercially available oil suspension (n = 22) or similar oil drops without the probiotic (n = 22). Parents recorded the frequency of bowel movements, stool consistency, inconsolable crying episodes, and any use of enemas; at baseline and at weeks 2, 4, and 8 the infants were examined by a physician.

Infants receiving *L. reuteri* DSM 17938 showed statistically significant increases in the frequency of bowel movements over the 8 weeks, and bowel movements were significantly more frequent in the probiotic group than the placebo group. Stool hardness decreased significantly from baseline to week 8 in the probiotic group, but stool hardness did not differ significantly between the probiotic and control groups. There were no differences in the frequency of inconsolable crying, and no adverse effects such as vomiting, bloating, or flatulence were reported. The authors concluded that "this prospective, placebo-controlled, double-blind study demonstrates the efficiency and safety of *L reuteri* DMS 17938 in infants with functional constipation to increase stool frequency."

Savino et al. (2010) studied the effect of *L. reuteri* strain DSM 17938 on infants exhibiting signs of colic in a randomized, double-blind, placebo-controlled trial. Fifty exclusively breastfed colicky infants (median age = 30.5 days) were randomly assigned (n = 25/group) to receive either 10^8 cfu *L. reuteri* strain DSM 17938 suspended in sunflower oil or placebo daily for 3 weeks. Growth parameters were recorded at baseline and on day 21; parents maintained diaries of crying time, stool frequency and characteristics, and any adverse effects such as constipation or vomiting. Fecal samples were obtained on day 21 for bacterial enumeration (*Lactobacillus, L. reuteri* DSM 17938, *Bifidobacterium, E. coli*, and *C. butyricum*) and analysis of ammonia.

All 25 infants receiving *L. reuteri* completed the study, but 4 infants discontinued from the control group—2 for compliance failure, 1 for fever, and 1 for gastroesophageal reflux. These were not regarded as trial-related adverse events. There were no differences between the groups in growth as measured by body weight, length, or head circumference. The infants receiving *L. reuteri* had significant increases in fecal counts of lactobacilli and significant decreases in ammonia and *E. coli* as compared to controls; no differences were seen in bifidobacteria or *C. butyricum*. *L. reuteri* DSM 17938 was detected in fecal samples from all but 1 of the infants in the probiotic group and in none of the samples from the control infants. Crying was significantly reduced in infants receiving *L. reuteri* DSM 17938 as compared to controls. The authors concluded, "*L. reuteri* DSM 17938 at a dose of 10^8 colony-forming units per day in early breastfed infants improved symptoms of infantile colic and was well tolerated and safe."

Continuing their research into the effects of *L. reuteri* strain DSM 17938 on gastrointestinal motility in infants, Indrio et al. (2011) randomized 42 infants with regurgitation to consume 10^8 cfu *L. reuteri* DSM 17938/day suspended in oil or placebo (21 infants/group) for 30 days in a randomized, double-blind, placebo-controlled study. The study population was drawn from otherwise healthy infants at least 3 weeks of age referred to the Gastrointestinal Unit for uncomplicated infant regurgitation, defined as regurgitation 2 or more times per day for 3 or more weeks. Twenty-one infants matched for age and weight but with no history of regurgitation

were recruited to establish the normal range of gastric emptying. Parents maintained diaries of the number of episodes of regurgitation and any other symptoms possibly related to the intervention. Gastric emptying was measured by ultrasound at the beginning and end of the 30-day intervention period.

Three infants from the probiotic group and 5 control infants were removed from the analysis due to noncompliance or withdrawal from the study, no withdrawals were due to adverse effects. As compared with control infants, those receiving *L. reuteri* DSM 17938 had significantly less frequent regurgitation and more rapid gastric emptying. There were no differences in body weight, length, or head circumference and no adverse events were reported related to the trial. The authors concluded, "In infants with functional GER [gastroesophageal reflux], *L. reuteri* DSM 17938 reduce[s] gastric distension and accelerate[s] gastric emptying. In addition, this probiotic strain seems to diminish the frequency of regurgitation."

Papagaroufalis et al. (2011; to be published) assessed the safety of powdered routine infant formula with 10^6 cfu *L. reuteri* DSM 17938/g powder. Eighty-eight healthy term infants were enrolled in a prospective, randomized, double-blind, controlled, multi-center trial during their first 2 days of life, and assigned to receive routine powdered whey-based infant formula providing either 0 or 10^8 cfu *L. reuteri*/day for 4 weeks (n = 44/arm). Morning urine was sampled and tested for D- and L-lactate and creatinine at baseline and at 1, 2, and 4 weeks; growth was assessed at these same time points based on measures of weight, length, and head circumference; 3-day diaries were collected in which parents reported sleep duration, stool characteristics, and such intolerance signs as crying, flatulence, spit-ups, or vomiting; and any adverse events were reported and evaluated. Blood was taken at 2 weeks for measurement of pH and acid/base excess.

Seventeen infants failed to complete the study, 9 from the control group and 8 from the *L. reuteri* group, none for reasons attributed to feeding. Compliance was excellent with no significant difference in formula consumption between the 2 groups. Both groups showed normal growth based on all 3 anthropomorphic measures, with no differences between infants receiving *L. reuteri*-supplemented formula and control formula. Urinary L-lactate concentrations did not differ between the 2 groups at any measured time point (0, 1, 2, and 4 weeks). D-lactate levels showed significantly different time trends between the groups, with the control groups showing a constant concentration through day 14 followed by an increase at day 28 and the *L. reuteri* group showing an increase at day 7, followed by a decrease through day 28. As a result, the median urinary D-lactate concentrations of the *L. reuteri* group were statistically significantly higher than the controls at 7 and 14 days and lower at baseline and 28 days. In no individual in either group did urinary D-lactate levels exceed a concentration 30-fold lower than the lowest level ever reported in short-bowel patients with symptomatic D-lactic acidosis (Perlmutter et al. 1983).

No differences were seen between groups in blood pH or acid/base excess at day 14, and no individual infant exhibited measures out of the normal range. Intolerance measures such as sleep interruptions, crying, spitting, vomiting, and flatulence were generally similar between the two groups; diarrhea was not reported in either group. There were no remarkable differences between the groups in reported adverse events; 7 infants in the control group and 6 in the *L. reuteri* group experienced minor adverse events while serious adverse events were reported for 2 control and 1 test infant.

Infant Studies Completed Since Preparation of GRN 254.

Reference	Objective	Study Design	Subjects	Probiotic and Dose	Duration	Safety-Related Results
Coccorullo et al. (2010)	Study the effects of ingestion of *L. reuteri* DSM 17938 on infants with functional chronic constipation	Randomized, double-blind, placebo-controlled trial	44 infants aged 6 months or more (mean age = 8.2 months) admitted to the GI Endoscopy and Motility Unit with chronic constipation	10^8 cfu *L. reuteri* DSM 17938/day in 5 drops of oil	8 weeks	Infants receiving *L. reuteri* DSM 17938 showed significant increases in the frequency of bowel movements, which were significantly more frequent in the probiotic group than the placebo group. Stool hardness decreased significantly in the probiotic group but did not differ between the probiotic and control groups. There were no differences in the frequency of inconsolable crying, and no adverse effects such as vomiting, bloating, or flatulence were reported. The authors concluded that "this prospective, placebo-controlled, double-blind study demonstrates the efficiency and safety of *L reuteri* DMS 17938 in infants with functional constipation to increase stool frequency."
Haschke-Becher et al. (2008)	Study urinary excretion of D- and L-lactate by infants ingesting *L. johnsonii* La1 in formula.	Randomized, double-blind, placebo-controlled trial with a breastfed reference group	71 healthy term infants (average age = about 106 days)	10^8 *cfu L. johnsonii* La1/g formula powder; daily intake of 0.8-1.1×10^{10} cfu	4 weeks	There were no differences in formula intake between the 2 formula groups nor among the 3 groups in growth. There were no differences in urinary D-lactate concentrations among the 3 groups at baseline, but after 4 weeks D-lactate excretion increased significantly in both formula groups as compared to the breastfed group, but the 2 formula groups did not differ in D-lactate excretion. There were no differences among the groups in L-lactate excretion at any time. The authors concluded that "current evidence does not point at any risk of lactate acidosis in healthy infants fed a formula supplemented with the probiotic strain La1."

Infant Studies Completed Since Preparation of GRN 254.

Reference	Objective	Study Design	Subjects	Probiotic and Dose	Duration	Safety-Related Results
Indrio et al. (2009)	Study the effects of GOS-FOS or *L. reuteri* DSM 17938 on GI motility in healthy preterm neonates	Randomized, double-blind, placebo-controlled trial	49 healthy preterm infants <1 week old	10^8 cfu *L. reuteri* DSM 17938/day	30 days	There were no differences between groups in growth or in reported adverse events. The breastfed group and infants receiving either the pre- or probiotic-supplemented formulas had significantly better EGG recordings and faster gastric emptying compared to the placebo group. The authors concluded that, "Feeding preterm infants with a formula supplemented with prebiotics or probiotics may stimulate gastric emptying and improve maturation of the EGG activity mimicking the effect of breast milk."
Indrio et al. (2011)	Study the effects of *L. reuteri* strain DSM 17938 on gastrointestinal motility in infants with gastro-esophageal reflux	Randomized, double-blind, placebo-controlled study	42 healthy infants at least 3 weeks of age with uncomplicated infant regurgitation	10^8 cfu *L. reuteri* DSM 17938/day suspended in 5 drops of oil	30 days	There were no withdrawals due to adverse effects. As compared with control infants, those receiving *L. reuteri* DSM 17938 had less frequent regurgitation and more rapid gastric emptying. There were no differences in body weight, length, or head circumference and no adverse events were reported related to the trial. The authors concluded, "In infants with functional GER, *L. reuteri* DSM 17938 reduce[s] gastric distension and accelerate[s] gastric emptying. In addition, this probiotic strain seems to diminish the frequency of regurgitation."
Papagaroufalis et al. (2011; to be published)	Assess the safety of infant formula with 10^6 cfu *L. reuteri* DSM 17938/g	Randomized, double-blind, controlled, multi-center trial	44 healthy term neonates during their 1st 72 hours of life	10^8 cfu *L. reuteri* DSM 17938/day	28 days	9 control and 8 test infants dropped out, none for reasons attributed to feeding. Both groups showed normal growth. Urinary L-lactate levels did not differ between the 2 groups. Median urinary D-lactate levels of the *L. reuteri* group were higher than the controls at 7 and 14 days and lower at baseline and 28 days. No infant had D-lactate levels exceeding 30-fold lower than the lowest level reported in symptomatic D-lactic acidosis. No differences or abnormalities were seen in blood pH or acid/base excess. Diarrhea was not reported and intolerance measures and adverse events were similar between the two groups.

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Infant Studies Completed Since Preparation of GRN 254.

Reference	Objective	Study Design	Subjects	Probiotic and Dose	Duration	Safety-Related Results
Savino et al. (2010)	Study the effect of *L. reuteri* strain DSM 17938 on infants exhibiting signs of colic	Randomized, double-blind, placebo-controlled study	50 exclusively breastfed colicky infants (median age = 30.5 days)	10^8 cfu *L. reuteri* DSM 17938/day suspended in 5 drops of oil	21 days	All infants receiving *L. reuteri* completed the study. There were no differences between the groups in body weight, length, or head circumference. The infants receiving *L. reuteri* had increased fecal counts of lactobacilli and significant decreases in ammonia and *E. coli* as compared to controls; no differences were seen in bifidobacteria or *C. butyricum*. *L. reuteri* DSM 17938 was detected in fecal samples from all but 1 of the infants in the probiotic group and in none of the samples from the control infants. Crying was significantly reduced in infants receiving *L. reuteri* DSM 17938 as compared to controls. The authors concluded, "*L. reuteri* DSM 17938 at a dose of 10^8 colony-forming units per day in early breastfed infants improved symptoms of infantile colic and was well tolerated and safe."

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5. Safety Assessment and GRAS Determination

5.1. Introduction

This section presents an assessment that demonstrates that *Lactobacillus reuteri* strain DSM 17938 is safe, and is GRAS, for addition as a probiotic to routine powdered whey-based infant formula intended for term infants.

This safety assessment and GRAS determination entail two steps. In the first step, the safety of *L. reuteri* strain DSM 17938 under its intended conditions of use is demonstrated. Safety is established by demonstrating a reasonable certainty that the exposure of infants to *L. reuteri* strain DSM 17938 under its intended conditions of use is not harmful. In the second step, the intended use of *L. reuteri* strain DSM 17938 is determined to be GRAS by demonstrating that the safety of this product under its intended conditions of use is generally recognized among qualified scientific experts and is based on publicly available and accepted information.

5.2. Safety Evaluation

A large number of studies, in animals, human adults, human children, and human infants, individually and collectively demonstrated the safety of *L. reuteri* ATCC 55730, the parent strain of *L. reuteri* DSM 17938. This body of evidence was presented and evaluated in GRN 254, which—as has already been stated—is incorporated by reference. Despite this incorporation, the following four paragraphs regarding safety in infants are repeated from GRN 254:

> *L. reuteri* ATCC 55730 was well studied in infants, including neonates ingesting *L. reuteri* on their first day of life and also including both preterm and full-term infants, both healthy and suffering from severe disorders. Eleven studies including over 1200 infants have been published, with *L. reuteri* doses as high as 1.2×10^9 cfu/day (Weizman et al. 2005). In 2 of these studies (Abrahamsson et al. 2007 and Connolly et al. 2005), the duration of *L. reuteri* treatment was 12 months. In addition to healthy full-term infants, populations studied have included infants with family histories of allergies, infants with colic, healthy preterm infants, and preterm infants confined to a Neonatal Intensive Care Unit (NICU) with severe health conditions. No behavioral or gastrointestinal side effects were noted in any of these studies and there were no adverse effects on growth or development. The study by Betta et al. (2007) is particularly significant because the population studied, premature neonates confined to an NICU, included about 10% surgical patients and about 50% with central venous catheters, both conditions widely recognized as posing the greatest risks of bacteremia. No adverse effects of any kind were observed even with these extremely sensitive neonates receiving 10^8 cfu/day *L. reuteri*.
>
> It was shown (Roos and Rosander 2005, 2006; Melin et al. 2008) that the modified *L. reuteri* strains DSM 17686 and DSM 17938 are substantially equivalent to the *L. reuteri* ATCC 55730 parent strain. In its discussion of the proposed rule establishing a GRAS notification process, FDA (1997) quoted from a report of a joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) consultation (FAO/WHO 1996):

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> "... substantial equivalence embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e. the food or food component can be concluded to be as safe as the conventional food or food component). Account should be taken of any processing that the food or food component may undergo as well as the intended use and the intake by the population."

FDA then noted that, "FDA believes that in certain instances the concept of substantial equivalence may have applicability to the technical element of a GRAS determination." Thus, the history of safe consumption of *L. reuteri* ATCC 55730, along with its demonstrated safety in both animal experiments and human clinical trials with adults, infants, and children, provides strong evidence of the safety of its daughter strain DSM 17938.

No adverse events attributable to feeding were reported in the 4 studies of *L. reuteri* strain DSM 17938—including one study in preterm infants—completed and published since preparation of GRN 254, further confirming the safety of the strain.

With regard to the potential for ingestion of *L. reuteri* to cause D-lactic acidosis, it is widely believed that this condition results only in infants or children with severely impaired carbohydrate absorption, nearly always due to short-bowel syndrome. Normal humans are known to be capable of metabolizing adequate quantities of D-lactate to prevent its accumulating in the blood serum. No case of metabolic acidosis was reported in the 17 studies in which *L. reuteri* strain ATCC 55730 or DSM 17938 was administered to infants, including both term and preterm infants and both healthy and severely compromised infants. Additionally, 3 studies in which D-lactate concentration was directly measured demonstrate that ingestion of probiotic bacteria that produce D-lactic acid does not result in metabolic harm:

- Connolly et al. (2005), discussed in GRN 254, showed that daily ingestion of 10^8 cfu *L. reuteri* ATCC 55730 by infants from birth resulted in no elevation of blood levels of D-lactate at 6 or 12 months and no indications of metabolic acidosis;
- Haschke-Becher (2008) found that daily ingestion of about 10^{10} cfu *L. johnsonii* La1for 4 weeks beginning at about age 4 months did not increase the concentration of D-lactate in morning urine samples; and
- Papagaroufalis et al. (2011) demonstrated that daily ingestion of 10^8 cfu *L. reuteri* DSM 17938 beginning within the first 72 hours of life, although producing a statistically significant increase in urinary D-lactate at 7 days of age, did not increase D-lactate to a level more than 30-fold below levels seen in pathologic D-lactic acidosis and did not increase blood pH or acid/base excess; furthermore, urinary D-lactate levels at 14 days and 28 days did not differ from those of infants receiving control formula; and, finally, all infants showed normal growth throughout the 28-day feeding period.

Finally, the genomic analysis by O'Sullivan (2008) confirmed that strain DSM 17938 does not harbor either virulence or transferable antibiotic resistance genes. In summary, a large body of published evidence demonstrates the safety of the intended use of *L. reuteri* strain DSM 17938.

5.3. General Recognition of the Safety of L. reuteri

The proposed addition of *L. reuteri* strain DSM 17938 as a probiotic to routine powdered whey-based infant formula intended for term infants has been determined to be safe through scientific procedures as set forth under 21 CFR §170.30(b). This safety was shown by establishing the identity and probiotic characteristics of the strain; demonstrating its freedom from pathogenic, toxigenic, antibiotic resistance, or other risk factors; finding that it does not lead to adverse metabolic effects; and concluding that the expected exposure to *L. reuteri* DSM 17938 by healthy term infants is without significant risk of harm. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of the addition of *L. reuteri* to infant formula has been made through the deliberations of an Expert Panel consisting of William J. Klish, M.D., Berthold V. Koletzko, M.D., Walter A. Mihatsch, M.D., Mary Ellen Sanders, Ph.D., Daniel J. O'Sullivan, Ph.D., Kelly A. Tappenden, Ph.D., and John A. Thomas, Ph.D., who reviewed a monograph prepared by JHeimbach LLC as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of infant formula and ingredients, including probiotic bacteria. They critically reviewed and evaluated the publicly available data, related information, and potential exposure to *L. reuteri* DSM 17938 anticipated to result from its intended uses, and determined that no evidence exists in the available information on *L. reuteri* DSM 17928, its parent strain ATCC 55730, other *L. reuteri* strains, or other probiotic bacteria, that demonstrates or suggests reasonable grounds to suspect a hazard to infants under the intended conditions of use of *L. reuteri* DSM 17938 in routine powdered whey-based infant formula intended for term infants.

5.4. Conclusion of the Expert Panel

We, the undersigned independent Expert Panel members, individually and collectively critically evaluated the data and information summarized above, and unanimously conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey based infant formula is safe under the following conditions of use: the microorganism is produced in accordance with current Good Manufacturing Practice and meets appropriate food-grade specifications; the microorganism is added at a minimum level of 10^8 colony-forming units/g powder with a target daily intake of 10^8 cfu; and the infant formula is intended for consumption by generally healthy non-immunocompromised term infants.

We further conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula under the conditions of use described above is generally recognized as safe.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

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We, the undersigned independent Expert Panel members, individually and collectively critically evaluated the data and information summarized above, and unanimously conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula is safe under the following conditions of use: the microorganism is produced in accordance with current Good Manufacturing Practice and meets appropriate food-grade specifications; the microorganism is added at a minimum level of 10^7 colony-forming units/g powder with a target daily intake of 10^8 cfu; and the infant formula is intended for consumption by generally healthy non-immunocompromised term infants.

We further conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula under the conditions of use described above is generally recognized as safe.

It is our unanimous opinion that other qualified experts would concur with these conclusions.

6 November 2011 (b) (6)

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We further conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula under the conditions of use described above is generally recognized as safe.

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We further conclude that the addition of *Lactobacillus reuteri* strain DSM 17938 to routine powdered whey-based infant formula under the conditions of use described above is generally recognized as safe.

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The reference list from GRN 254 is incorporated by reference. Additional references are listed below.

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