SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related Immunofortis® and strengthening of the baby's immune system pursuant to Article 14 of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

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ABSTRACT

Following an application from Danone Baby Nutrition, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of The Netherlands, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to Immunofortis® to naturally strengthen the baby's immune system. The scope of the application was proposed to fall under a health claim referring to children's development and health The Panel considered that the food constituent, Immunofortis®, a 9:1 mixture of short-chain galacto- and long-chain fructo-oligosaccharides, was sufficiently characterised. The target population consisted of infants who were not breastfed or who were partially breastfed and who were less than 12 months old. The Panel considered that a well-functioning immune system includes the initiation of appropriate adaptive immune responses and an appropriate defence against pathogens and was a beneficial physiological effect.

In weighing the evidence, the Panel took into account that the one human intervention study investigating the effects of Immunofortis® on the incidence of atopic dermatitis and the overall cumulative incidence of infections had considerable limitations, that the evidence for an effect of Immunofortis® on the reduction of potentially pathogenic bacteria was inconsistent, that the evidence for an effect of Immunofortis® on immune function animal studies did not predict the occurrence of an effect in humans, and that the evidence presented in support of a biologically plausible mechanism by which Immunofortis® could exert the claimed effect was not convincing.

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[1] On request from Danone Baby Nutrition, The Netherlands. Question No EFSA-Q-2008-106, adopted on 04 December 2009.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims for the preparation of this opinion: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren, Hans Verhagen and Susanne Krauss-Etschmann, and EFSA staff member Wolfgang Gelbmann for the support provided to this EFSA scientific output.

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The Panel concluded that the evidence provided was insufficient to establish a cause and effect relationship between the consumption of Immunofortis® and the claimed effect of initiation of appropriate immune responses including the defence against pathogens.

KEY WORDS

(Galacto-oligosaccharides, fructo-oligosaccharides, Immunofortis®, infections, allergy, infants, infant formula).

SUMMARY

Following an application from Danone Baby Nutrition, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of The Netherlands, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to Immunofortis® to naturally strengthen the baby's immune system.

The scope of the application was proposed to fall under a health claim referring to children's development and health and including a request for the protection of proprietary data.

The Panel considers that the food constituent, Immunofortis®, a 9:1 mixture of short-chain galacto- (scGOS) and long-chain fructo-oligosaccharides (lcFOS), is sufficiently characterised.

The claimed effect is "to naturally strengthen the baby's immune system". The target population consists of infants who are not breastfed or who are partially breastfed and who are less than 12 months old. In the context of the proposed health relationship, the Panel assumes that the claimed effect refers to a reduction in the risk of "allergic symptoms and common infections". The Panel considers that a well-functioning immune system includes the initiation of appropriate adaptive immune responses and an appropriate defence against pathogens. The Panel considers that the initiation of appropriate immune responses including the defence against pathogens is a beneficial physiological effect.

The applicant provided 25 study references on human data and 5 references on non-human studies. In addition, the applicant submitted 23 references which included publications of the category reviews, guidelines/consensus opinion and text book chapters.

In a double-blind randomised controlled trial (RCT) with 259 infants with a parental history of atopic dermatitis (AD), allergic rhinitis or asthma, the effects of an extensively hydrolysed cow's milk whey protein formula with 8 g/L scGOS/lcFOS (9:1 mixture, Immunofortis®) or with the same amount of maltodextrin in the placebo group on the incidence of atopic dermatitis and infections were studied. At 6 months of age 206 infants completed the study. A significantly lower number of infants had developed AD by the end of the intervention in the Immunofortis® group (10 out of 102 infants; 9.8 %; 95 %CI 5.4 – 17.1) than in the control group (24 out of 104 infants; 23.1 %; 95 %CI 16.0 – 32.1), whereas the severity of AD in the Immunofortis® group did not differ significantly from that in the placebo group. Infants in the Immunofortis® group had significantly fewer episodes of all types of reported infections combined than those in the control group (21 vs. 47), corresponding to a statistically significantly lower cumulative incidence of infections (16 % vs. 29 %; $p < 0.05$). The number and cumulative incidence of upper respiratory tract infections, otitis media, gastrointestinal or urinary tract infections did not differ statistically between groups, nor did the number of prescribed antibiotic treatments. At the age of 2 years, 134 children (52 % of the initial 259) finished the follow up period. In the Immunofortis® group the cumulative incidence of AD (13.6 % vs. 27.9 %; $p < 0.05$), recurrent wheeze (8 % vs. 21 %, $p < 0.05$), and allergic urticaria (1.5 % vs. 10 %; each $p < 0.05$) was significantly lower than in the control group. The number per infant of all types of paediatrician-diagnosed infections [4.1 ± 3.1 vs. 5.9 ± 4.1 (mean ± SD); $p < 0.01$], of upper respiratory tract infection (3.2 ± 2.2 vs. 2.1 ± 1.8; $p < 0.01$), of fever episodes reported by the parents (2.2 ± 1.9 vs. 3.9 ± 2.5; $p < 0.0001$) and of antibiotic prescriptions (1.8 ± 2.3 vs. 2.7 ± 2.4; $p < 0.01$) was significantly lower in the Immunofortis® group than in the control group. There was no statistically significant difference between groups in the number of otitis media, lower respiratory tract infections, gastrointestinal and urinary tract infections.

The Panel notes a number of weaknesses in this study: the "allergic" nature of clinically diagnosed dermatitis, wheezing and urticaria were not assessed (i.e., by the measurement of commonly accepted immunological parameters); it is unclear how the diagnosis of infection was made by primary care paediatricians (clinical signs and/or symptoms vs. microbiological/serological diagnosis in biological

samples), what were the criteria used by paediatricians for antibiotic prescription, and whether those criteria where applied uniformly in the intervention and control groups; there was also a lack of a correction for multiple testing considering the high number of endpoints measured. The Panel considers that the considerable weaknesses of this study, including its follow-up, limit its value as a source of data to support the claimed effect.

The Panel considers that no scientific conclusions can be drawn for substantiation of the claim from RCTs investigating clinical end-points that were either performed with a formulation that included pectin derived acidic oligosaccharides and was therefore different from Immunofortis®, or that had limitations in study design and/or were lacking details of study methods and analysis.

The applicant provided 5 double-blind randomised, placebo controlled studies investigating the effect of an infant milk formula with or without scGOS/lcFOS on the number/proportion of various potentially pathogenic bacteria in the stool flora of infants. The Panel considered that for four of these studies the evidence provided does not establish that outcomes measured are appropriate endpoints for a reduction of potentially pathogenic bacteria and that no scientific conclusion can be drawn from these studies regarding the effect of scGOS/lcFOS on the reduction of potentially pathogenic bacteria in the intestine. For the fifth study, the Panel considers that the evidence for a reduction in potentially pathogenic bacteria was inconsistent.

The Panel considers that the evidence provided on effects of Immunofortis® on immune function in five animal studies submitted does not predict the occurrence of an effect of Immunofortis® on the initiation of appropriate immune responses including the defence against pathogens in humans.

The applicant proposed a number of possible mechanisms by which Immunofortis® could exert the claimed effect. A number of studies showed a significant increase of the number/proportion of bifidobacteria in stool samples of infants after consumption of scGOS/lcFOS. The Panel considers that the evidence provided does not establish that an increased number and/or proportion of bifidobacteria in faeces represent a mechanism for initiation of appropriate immune responses. In addition, possible roles for the development of an anti-allergic immunoglobulin profile, or changes in plasma immunoglobulin free light-chain (Ig-fLC) concentrations, or an increase in sIgA, are not supported by the evidence provided.

In weighing the evidence, the Panel took into account that the one human intervention study investigating the effects of Immunofortis® on the incidence of atopic dermatitis and the overall cumulative incidence of infections had considerable limitations, that the evidence for an effect of Immunofortis® on the reduction of potentially pathogenic bacteria was inconsistent, that the evidence for an effect of Immunofortis® on immune function in animal studies does not predict the occurrence of an effect in humans, and that the evidence presented in support of a biologically plausible mechanism by which Immunofortis® could exert the claimed effect is not convincing.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of Immunofortis® and the initiation of appropriate immune responses including the defence against pathogens.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1924/20064 harmonises the provisions that relate to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of that Regulation and are authorised in accordance with this Regulation and included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of that Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of that Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, who will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

The application was received on 29/01/2008.

The scope of the application was proposed to fall under a health claim referring to children's development and health.

During the check for completeness[5] of the application, the applicant was requested to provide missing information on 11/03/2008.

The applicant provided the missing information on 01/04/2009.

The scientific evaluation procedure started on 15/04/2009.

On 15/09/2009, the NDA Panel agreed on the List of Questions which requests the applicant to supplement additional particulars to accompany the application.

The applicant submitted the responses to the NDA Panel List of Questions on 29/10/2009.

During the meeting on 04/12/2009, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to Immunofortis® and the baby's immune system.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: Immunofortis® and the baby's immune system.

[4] European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

[5] In accordance with EFSA "Scientific and Technical guidance for the Preparation and Presentation of the Application for Authorisation of a Health Claim"

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of Immunofortis®, a positive assessment of its safety, nor a decision on whether Immunofortis® is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Danone Baby Nutrition, WTC Schiphol Airport, Tower E, Schiphol Boulevard 105, 1118 BG Schiphol Airport, The Netherlands.

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006. The applicant claims the food composition, the manufacturing process and the clinical studies conducted with Immunofortis® as being proprietary.

Food/constituent as stated by the applicant

A prebiotic mixture of short chain galacto-oligosaccharides (hereafter called scGOS) and long chain fructo-oligosaccharides (hereafter called lcFOS) added to formulae for infants up to the age of 12 months in a ratio of 9:1. The prebiotic mixture is composed of 90 % low molecular weight scGOS and 10 % high molecular weight lcFOS intended to mimic the prebiotic effect of human milk oligosaccharides including amount and size distribution of molecules. The scGOS are milk based ingredients and the lcFOS are derived from chicoree inulin.

The applicant refers to this ingredient by the registered trade mark of "Immunofortis®".

Health relationship as claimed by the applicant

According to the applicant, there is evidence that the intestinal microbiota plays a key role in the postnatal development of the immune system. Natural colonisation of the infant's gut starts with bacteria mainly from the vaginal and intestinal microbiota of the mother. Diet plays an important role in the further development of the intestinal microbiota of the infant. During breastfeeding the composition of the gut microbiota develops within a short period and becomes dominated by bifidobacteria, whereas formula fed infants develop a microbiota of a more adult type. The intestinal ecosystem is thought to be fairly established around 2 years of age. The applicant contends that via the change in gut microbiota and/or via stimulation of unique receptor systems on immunocompetent cells, the scGOS/lcFOS mixture leads to a reduced risk of allergic symptoms and common infections, confirming an effective natural strengthening of the immune system of infants.

Wording of the health claim as proposed by the applicant

Immunofortis® to naturally strengthen your baby's immune system.

Specific conditions of use as proposed by the applicant

The target population consists of infants which are not breastfed or partially breastfed and who are less than 12 months old. Pursuant to the restriction laid down in Directive 2006/141/EC on infant and follow-on formulae with regard to the use of health claims on infant formula, the *claim* is only to be made on follow-on formulae and formulae for infants falling under Directive 1999/21/EC on foods for special medical purposes.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is Immunofortis®, a mixture of short-chain ß-galacto- and long-chain fructo-oligosaccharides (scGOS: glucose (galactose)$_{1\text{-}7;}$ glucose (fructose)$_{1\text{-}60}$ (Average 23) at a scGOS : lcFOS ratio of 9:1 that is used as a food ingredient in infant and follow-on formulae. To obtain scGOS, a lactose syrup is produced from whey and transformed by ß-galactosidases into scGOS. Long-chain FOS are obtained from fractionation of inulin prepared from chicory roots. Both final preparations are stable over the shelf life of the final (reconstituted) infant and follow-on formula. They are added at a total concentration of 0.8 g/100 ml in the reconstituted formula (scGOS 0.72 g/100 ml)

The Panel considers that the food constituent, Immunofortis®, a 9:1 mixture of short-chain galacto- (scGOS) and long-chain fructo-oligosaccharides (lcFOS), that is the subject of the health claim is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "to naturally strengthen your baby's immune system". The target population consists of infants who are not breastfed or are only partially breastfed and who are less than 12 months old.

In the context of the proposed health relationship, the Panel assumes that the claimed effect refers to a reduction in the risk of "allergic symptoms and common infections". The Panel considers that a well-functioning immune system includes the initiation of appropriate adaptive immune responses and an appropriate defence against pathogens.

The Panel considers that the initiation of appropriate immune responses including the defence against pathogens is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant searched electronic databases (Medline, Current Contents, Biosis, SciSearch, CAB Abstracts, Food Sci and Tech Abstracts, Embase) using keywords related to infants, bifidobacteria, lactobacilli, microbiota, faeces, T-helper cells, immune system, immunoglobulin, atopy, allergy, galacto-and fructo-oligosaccharides.

The applicant identified 30 references as pertinent to the claim, including 25 references reporting on human data and five references on non-human studies. The human studies included 18 publications and four unpublished manuscripts/study reports on randomised controlled trials (RCT), two published observational human studies, and one publication in the category "other human studies". The non-human data included four published animal studies and one unpublished *ex vivo/in vitro* study. In addition, the applicant made reference to 18 review articles, four publications in the category "guidelines/consensus opinions/text book chapters" and one publication classified by the applicant as "other".

A report was provided on a multi-centre, double-blind RCT which tested the combination of 0.68 g scGOS/lcFOS (9:1) plus 0.12 g pectin derived acidic oligosaccharides (pAOS) added to a standard non-hydrolysed cow's milk based formula at a concentration of 0.8 g/dL (Frings, 2009, unpublished). Since data provided by the applicant suggest that pAOS, when added to the combination of scGOS:lcFOS in Immunofortis®, may have an impact on immune responses in animals (Vos et al., 2007a and 2007b) and on the numbers of intestinal bifidobacteria in infants (Magne et al., 2008), and

both modulation of immune responses as well as modulation of intestinal bifidobacteria have been advocated by the applicant as the mechanisms by which Immunofortis® could exert the claimed effect (see below), the Panel considers that no scientific conclusions can be drawn from studies conducted with scGOS/lcFOS (9:1) plus pAOS (Frings, 2009, unpublished; one study arm of Magne et al., 2008; Westerbeek et al., 2008; 2009a; 2009b) to substantiate the claimed effect on Immunofortis®. Similarly, no scientific conclusions can be drawn to substantiate the claimed effect on Immunofortis® from the studies in infants conducted with the combination of scGOS/lcFOS plus pAOS Bruzzese et al. (2009) performed an open-label RCT where 342 healthy infants 15 - 120 days of age were enrolled by 38 practitioners in seven different Italian regions. Children received a standard formula with or without Immunofortis® at 0.4 g/100 mL for 12 months from the time when exclusive breastfeeding was stopped. Routine visits conducted every three months included a medical examination and further clinical assessment was performed in case of gastrointestinal or respiratory symptoms. Primary endpoints were incidence of acute diarrhoea, of lower and upper respiratory tract infections (URTI), and the number of antibiotic courses prescribed for respiratory infections. The Panel notes that diarrhoeal episodes in young infants do not necessarily reflect infection, and therefore no conclusions can be drawn from this outcome for the substantiation of the claimed effect. The dropout rate was 43 % in the Immunofortis® group and 39 % in the control group owing to "loss for follow-up", protocol violation and missing data. A per-protocol analysis was performed on the 201 infants who completed the study (96 in the Immunofortis® group). There were no statistically significant differences between groups in the number of episodes of lower and URTI. The number of infants receiving two or more antibiotic courses for respiratory infections was statistically significantly lower in the Immunofortis® group than in the control group (24/60 vs. 53/65). The Panel notes a number of weaknesses in this study: the open-label design, the high dropout rate, the broad age range at inclusion, and the lack of information on the duration of partial breastfeeding. Further, it is unclear from the publication how the diagnosis of infection was made (clinical signs and/or symptoms vs. microbiological diagnosis in biological samples), what were the criteria used by paediatricians for antibiotic prescription, and whether those criteria were applied uniformly in the intervention and control groups. The Panel considers that no scientific conclusions can be drawn from this study for the substantiation of the claimed effect.

An abstract of an oral presentation was provided reporting on a study in 160 term infants receiving a standard infant formula with or without a 9:1 scGOS/lcFOS mixture (Nyankovskyy et al., 2009). However, the data provided by this abstract are very limited and do not include information on inclusion/exclusion criteria, power calculations, statistical analysis, dropout rates, or definition of study endpoints. The Panel considers that no scientific conclusions can be drawn from this abstract for the substantiation of the claimed effect.

One double-blind RCT in the target population (i.e., infants) was presented using an infant formula containing the food constituent for which the claim is made (Immunofortis®) at the proposed conditions of use (0.8 g/dL). Three publications report on clinical outcomes from this study and its follow up directly related to the claimed effect ("allergic symptoms" and "common infections" as surrogate markers of the initiation of appropriate immune responses including the defence against pathogens) (Moro et al., 2006; Arslanoglu et al., 2007; Arslanoglu et al., 2008).

A total of 259 infants (128 males) were recruited for the study by applying the following inclusion criteria: born at term (gestational age between 37 and 42 weeks), with an appropriate weight for gestational age, starting formula feeding within the first two weeks of life, and having a parental history of atopic dermatitis (AD), allergic rhinitis or asthma (Moro et al., 2006). Infants were randomly assigned to receive an extensively hydrolysed cow's milk whey protein formula with either 8 g/L scGOS/lcFOS (9:1 mixture, Immunofortis®; n = 129) or the same amount of maltodextrin (control; n = 130) for six months following a double-blind, randomised, controlled design. Infants breastfed beyond the 6th week of life in addition to the per-protocol infant formulae were excluded from the study. Primary endpoints were the cumulative incidence and severity of atopic dermatitis (AD) during the first 6 months of life (Moro et al., 2006), the number of infectious episodes (overall,

URTI, otitis media, gastrointestinal infections, and urinary tract infections), the number of infections requiring antibiotic therapy, the cumulative incidence of one or more (recurrent) infectious episodes in the first six months of life, and the incidence of infectious episodes at different time points as assessed by parental interview during monthly visits and physicians' documentation (Arslanoglu et al., 2007). AD was clinically diagnosed by two investigators based on the presence of all of the following: pruritus, involvement of the face, skull, facial and/or extensor part of the extremities, and a minimal duration of the symptoms of 4 weeks. Severity of AD was assessed using the "SCORing Atopic Dermatitis" (SCORAD) scheme. How the diagnoses of infections were made by primary care paediatricians (clinical signs and/or symptoms vs. microbiological diagnosis in biological samples), which criteria were used by primary care paediatricians for antibiotic prescription, and whether those criteria were applied uniformly was not reported (Arslanoglu et al., 2007). The sample size calculation was based on the previous year's incidence of AD in the study hospital assuming a 50 % difference in the incidence of AD between groups and a drop out rate of 15% (Moro et al., 2006). In order to achieve a power of 80 % with an $\alpha < 0.05$, 108 infants per group completing the study were needed. As 53 (20 %) infants dropped out, mostly because of continued breastfeeding, only 102 (52 male) and 104 (49 male) infants completed the 6-month intervention period in the Immunofortis® and the control groups, respectively. Statistical analyses on the outcome variables were only performed for the per-protocol population.

No differences for a number of baseline characteristics were reported between the Immunofortis® and control groups. A significantly lower number of infants developed AD by the end of the intervention in the Immunofortis® group (10 out of 102 infants; 9.8 %; 95 %CI 5.4 – 17.1) than in the control group (24 out of 104 infants; 23.1 %; 95 %CI 16.0 – 32.1), whereas the severity of AD in infants in the Immunofortis® group did not differ significantly from that in infants in the placebo group (Moro et al., 2006). Infants in the Immunofortis® group had significantly fewer episodes of all types of reported infections combined than those in the control group (21 vs. 47), corresponding to a statistically significantly lower cumulative incidence of infections (16 % vs. 29 %; $p < 0.05$). The number (or cumulative incidence) of URTI, otitis media, gastrointestinal or urinary tract infections did not differ statistically between groups, nor did the number of prescribed antibiotic treatments.

Parental agreement to participate in a double-blinded follow-up until the infants were two years of age was obtained for 152 infants (Arslanoglu et al., 2008). The follow-up visits at 9, 12, 18 and 24 months of age consisted of physical examinations, evaluation of growth and interviews of the parents by the study physicians. Parents were instructed to report symptoms potentially related to allergic reactions (eczema, wheezing episodes, urticaria) and infections (episodes of fever, runny nose, and watery stools) and to provide information on clinical visits, primary care physician's diagnoses, tests and medical prescriptions. Primary endpoints were the cumulative incidences of allergic manifestations (AD, recurrent wheezing and allergic urticaria) until the age of 24 months. Diagnosis and severity of AD were assessed as described for the 6-month study period (Moro et al., 2006). Recurrent wheezing was defined as ≥ 3 physician diagnosed wheezing episodes. Allergic urticaria was defined as two or more episodes of itching eruptions or swelling with typical appearance provoked by the same allergen. Secondary outcomes were the number of physician-diagnosed infections, fever (> 38.5° C) witnessed by parents, the number of antibiotic prescriptions, and growth. Physician-diagnosed infections included URTI, lower respiratory tract infections, otitis media, and gastrointestinal infections. No sample size calculation was performed for the follow-up study.

One-hundred thirty-four infants (66 in the Immunofortis® group) completed the follow-up at 2 years of age. The overall drop-out rate owing due to poor compliance was 12 % (n=18). A per-protocol analysis was conducted. Baseline characteristics of completers did not differ significantly between groups. In the Immunofortis® group the cumulative incidence of AD (13.6 % vs. 27.9 %; $p < 0.05$), recurrent wheeze (8 % vs. 21 %, $p < 0.05$), and allergic urticaria (1.5 % vs. 10 %; $p < 0.05$) were significantly lower than in the control group. The number per infant of all types of paediatrician-diagnosed infections (mean ± SD =4.1 ± 3.1 vs. 5.9 ± 4.1; $p < 0.01$), of URTI (3.2 ± 2.2 vs. 2.1 ± 1.8; $p < 0.01$), of fever episodes reported by the parents (2.2 ± 1.9 vs. 3.9 ± 2.5; $p < 0.0001$) and of

antibiotic prescriptions (1.8 ± 2.3 vs. 2.7 ± 2.4; $p < 0.01$) was significantly lower in the Immunofortis® group than in the control group. There were no statistically significant differences between groups in the number of otitis media, lower respiratory tract infections, gastrointestinal and urinary tract infections. Less than 53.7 % of the paediatricians-diagnosed infections in the intervention group and less than 66.1 % in the control group were accompanied by fever reported by the parents.

The Panel notes a number of weaknesses in this study: the "allergic" nature of clinically diagnosed dermatitis, wheezing and urticaria was not assessed (i.e., by the measurement of commonly accepted immunological parameters); it is unclear how the diagnosis of infection was made by primary care paediatricians (clinical signs and/or symptoms vs. microbiological/serological diagnosis in biological samples), what criteria were used by paediatricians for antibiotic prescription, and whether those criteria where applied uniformly in the intervention and control groups; there was a lack of a correction for multiple testing in the statistical analysis considering the high number of endpoints measured and reported (Arslanoglu et al., 2007 and 2008; Moro et al., 2006). The Panel considers that the considerable weaknesses of this study (including its follow-up) limit its value as a source of data to substantiate the claim.

The applicant also suggested that the prebiotic mixture of scGOS/lcFOS (9:1) may protect against the colonisation of potentially pathogenic bacteria and provided five references to support this claimed effect (Costalos et al., 2008; Knol et al, 2005a; Magne et al., 2008; Scholtens et al., 2008a; 2008b).

In a double blind RCT, Costalos et al. (2008) compared the median/range of the proportion of faecal E. coli and clostridia in the stool flora in samples from 64 bottle-fed infants (32 per group) randomised to receive a formula with or without 0.4 g scGOS/lcFOS (9:1). The proportions of E. coli were not significantly different between the intervention and control groups. The reported median of the proportion of faecal clostridia of the total microorganisms was initially 0 % for both groups (no ranges provided) and after 6 weeks of treatment was 0/0.00-67.04 % in the GOS/FOS group versus 3.29/0.00-29.29 % in the control group. Although the difference between groups at 6 weeks was reported to be significant ($p = 0.042$) this is not apparent from the data presented in the paper. The Panel notes the wide range of values observed, the marginal significance of the reported difference in medians between groups, and the lack of clarity in the data presented and considers that no scientific conclusions can be drawn from the study regarding the effect of scGOS/lcFOS on faecal clostridia.

Knol et al. (2005a) investigated the total number as well as the cumulative share (%) of a number of bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Pseudomonas aeruginosa, Enterobacter, Klebsiella, Proteus, Streptococcus group B, Clostridium difficile, Bacillus subtilis* and *Acinetobacter)* in faecal flora in 12 preterm infants fed with 1 g scGOS/lcFOS (9:1) per 100 mL formula compared to that of 13 infants fed with an un-supplemented control formula. The Panel notes that no data were provided on the individual species of bacteria and that the evidence provided does not establish that the sum of all bacteria measured is an appropriate endpoint for a reduction of potentially pathogenic bacteria. The Panel considers that no scientific conclusion can be drawn from this study regarding the effect of scGOS/lcFOS on the reduction of potentially pathogenic bacteria in the intestine.

Scholtens et al. (2008a) investigated in a double-blind RCT the effect of an infant milk formula with or without 6 g/L scGOS/lcFOS (9.1) on the percentage of *Clostridium spp*. and *E. coli* of the total faecal bacteria. Magne et al. (2008) investigated in a double-blind RCT the effect of a whey based formula with or without 6 g/L scGOS/lcFOS (9:1) on 7 bacterial groups in stool samples of 68 infants. The Panel notes that the evidence provided does not establish that changes in the proportions of these microorganisms are appropriate endpoints for a reduction of potentially pathogenic bacteria. The Panel considers that no scientific conclusion can be drawn from this study regarding the effect of scGOS/lcFOS on the reduction of potentially pathogenic bacteria in the intestine.

In another unpublished double-blind RCT (Scholtens et al., 2008b) 12 g/L scGOS/lcFOS (9:1) formula was fed to 62 infants at least for 6 weeks before the age of 12 months, and 4 weeks thereafter. The study investigated the percentage of bacteria of the *Clostridium histolyticum/C. lituseburense* group and of the *Eubacterium rectale*/*Clostridium coccoides* group of the total faecal bacteria at the age of 12 and 13 months. No statistically significant effects were seen for *Enterobacteriaceae* and *Bacteroides* at either time point and for *Eubacterium spp*. and *Clostridium spp.* at the age of 13 months. Statistically significant lower percentages were seen for *Clostridium spp*. and *Eubacteria spp*. at the age of 12 months but not at the age of 13 months. The Panel notes the inconsistency of the results.

Two mouse studies aimed to analyse the influence of scGOS/lcFOS on vaccine specific delayed type hypersensitivity (DTH) as a marker of Th1 immunity (Vos et al., 2006; 2007a). A third study by Vos et al. (2007b) investigated the effect of scGOS/lcFOS on several parameters of allergic asthma in the ovalbumin mouse model for asthma. Schouten et al. (2009b) studied the effect of Immunofortis® in mice on the allergic response when provided during oral sensitisation with whey. DeKivit et al. (2009) studied the effect of scGOS/lcFOS on the galectin-4 expression of human intestinal cells *in vitro* and the effect on the phenotype of co-cultured activated healthy donor peripheral blood mononuclear cells. The Panel considers that the evidence provided in the animal studies submitted does not predict the occurrence of an effect of Immunofortis® on the initiation of appropriate immune responses including the defence against pathogens in humans.

In addition, the applicant proposed a number of mechanisms by which Immunofortis® could exert the claimed effect (i.e., via the change in the gut microbiota and/or via direct stimulation of the immune system).

A number of studies showed a significant increase in the number/proportion of bifidobacteria in stool samples of infants after consumption of scGOS/lcFOS (Boehm et al., 2002; Bakker-Zierikzee et al. 2005; Costalos et al., 2007; Desci et al., 2005; Haarman and Knol 2005, 2006; Knol et al. 2005a, 2005b; Magne et al. 2008; Moro et al., 2002; 2005; 2006; Penders et al., 2006; Rinne et al 2005; Schmelze et al., 2003; Scholtens et al., 2008b). In two RCT, the supplementation of scGOS/lcFOS to infant formula showed an increase in the proportion (Haarman and Knol, 2006) or the number of lactobacilli (Moro et al., 2002) in the faecal microbiota. Such an effect was not observed in two previously described RCT including 102 and 120 subjects in the intervention group (Moro et al., 2006 and Scholtens et al., 2008b, respectively). The Panel considers that the evidence provided does not establish that an increased number/proportion of bifidobacteria or lactobacilli in faeces represents a mechanism for initiation of appropriate immune responses.

The applicant has also suggested that in subjects receiving scGOS/lcFOS, the reduction of allergic reactions in early life may occur through the development of an anti-allergic immunoglobulin profile induced by Immunofortis® and presented data from a study by van Hoffen et al. (2009) who investigated this suggested mechanism in a subgroup of the population studied by Moro et al. (2006). Van Hoffen et al. (2009) reported that plasma concentrations of total IgE, IgG1, IgG2 and IgG3, but not of IgG4, were statistically significantly lower in infants receiving Immunofortis®. However, this proposed mechanism is not supported by the observation that, within that subpopulation of infants with observed AD, immunoglobulin concentrations were not different from those in infants without AD.

Another possible mechanism proposed by the applicant was related to plasma immunoglobulin free light-chain (Ig-fLC) concentrations. Schouten et al. (2009a; unpublished) studied plasma Ig-fLC concentrations in a sub-group of 74 infants participating in the study by Moro et al. (2006). Plasma concentrations of kappa- and lambda Ig-fLC were significantly lower in the 34 infants receiving Immunofortis®, which included 6 cases of reported AD, than in the 40 infants receiving the control formula, which included 19 cases of reported AD. The Panel notes that no information has been provided on how (and on which basis) this sub-sample of subjects was selected from the original

study population, and considers that the evidence provided does not establish a mechanistic role of Ig-fLC in the pathogenesis of allergic diseases of infants.

Another possible mechanism suggested by the applicant is that a stimulation of the mucosal immune system may occur in subjects receiving Immunofortis® and that this is indicated by an increase in faecal sIgA; however, in two studies investigating the effect of Immunofortis® on faecal sIgA in infants the evidence for increased faecal sIgA is inconsistent (Bakker-Zieriksee et al., 2006; Scholtens et al., 2008a) and in one study the effect was statistically not significant (Scholtens et al., 2008b, unpublished).

In addition to human and non-human studies, the applicant provided 18 references including review articles, publications of the category "guidelines/consensus opinions" (e.g. immunological biomarkers) and text book chapters (e.g. on the effect of "prebiotic" oligosaccharides on the gut microbiota, microbes and the developing gastrointestinal tract, the bacterial colonisation of the intestine in breastfed infants, the positive effects of breastfeeding against infections, the application of "prebiotics" in infant foods, immune modulatory factors in human milk). The Panel considers that no scientific conclusions can be drawn from these references for the substantiation of the claimed effect on Immunofortis®.

In weighing the evidence, the Panel took into account that the one human intervention study investigating the effects of Immunofortis® on the incidence of atopic dermatitis and the overall cumulative incidence of infections had considerable limitations, that the evidence for an effect of Immunofortis® on the reduction of potentially pathogenic bacteria was inconsistent, that the evidence for an effect of Immunofortis® on immune function animal studies does not predict the occurrence of an effect in humans, and that the evidence presented in support of a biologically plausible mechanism by which Immunofortis® could exert the claimed effect is not convincing.

The Panel concludes that the information provided is insufficient to establish a cause and effect relationship between the consumption of Immunofortis® and the initiation of appropriate immune responses including the defence against pathogens.

CONCLUSIONS

- The food supplement which is the subject of the claim, Immunofortis®, a mixture of short-chain galacto- and long-chain fructo-oligosaccharides, is sufficiently characterised.
- The Panel considers that initiation of appropriate immune responses including the defence against pathogens is a beneficial physiological effect. The target population is infants up to 12 months of age.
- The information provided is insufficient to establish a cause and effect relationship between the consumption of Immunofortis® and the claimed effect of initiation of appropriate immune responses including the defence against pathogens.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on Immunofortis® and strengthening the baby's immune system pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0026_NL). 01/04/2009. Submitted by Danone Baby Nutrition.

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GLOSSARY / ABBREVIATIONS

AD	atopic dermatitis
lcFOS	long chain fructo-oligosaccharides
pAOS	pectin derived acidic oligosaccharides (pAOS)
RCT	randomised controlled trial
SCORAD	"SCORing Atopic Dermatitis" scheme
scGOS	short chain galacto-oligosaccharides
URTI	upper respiratory tract infections