SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 December 2016

doi: 10.2903/j.efsa.2017.4679

'Nutrimune®' and immune defence against pathogens in the gastrointestinal and upper respiratory tracts: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen-Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Martin Stern, Daniel Tomé, Henk Van Loveren, Marco Vinceti, Peter Willatts, Ambroise Martin, John J Strain and Alfonso Siani

Abstract

Following an application from H.J. Heinz Supply Chain Europe B.V., submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of the Netherlands, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to 'Nutrimune®' and immune defence against pathogens in the gastrointestinal (GI) tract and upper respiratory tract (URT). The food 'Nutrimune®' (a pasteurised cow's skim milk fermented with *Lactobacillus paracasei* CBA L74) which is the subject of the health claim is sufficiently characterised. The Panel considers that immune defence against pathogens in GI tract and URT is a beneficial physiological effect. One human intervention study from which conclusions can be drawn showed an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT, and the results from one animal study could support an effect of 'Nutrimune®' on defence against pathogens in the GI tract. However, there were inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI in the human intervention study, the results of this study have not been replicated, and no evidence was provided for a plausible mechanism by which 'Nutrimune®' could exert the claimed effect in vivo in humans. The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of 'Nutrimune®' and immune defence against pathogens in the gastrointestinal and upper respiratory tracts.

Keywords: Nutrimune®, immune defence, gastrointestinal tract, upper respiratory tract, infection, children, health claims

Requestor: Competent Authority of the Netherlands following an application by H.J. Heinz Supply Chain Europe B.V.

Question number: EFSA-Q-2016-00008

Correspondence: nda@efsa.europa.eu

Panel members: Jean Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J. McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Martin Stern, Daniel Tomé, Dominique Turck, Hendrik Van Loveren, Marco Vinceti and Peter Willatts.

Competing interests: In line with EFSA's policy on declarations of interest, Panel member Anders Sjödin did not participate in the development and adoption of this scientific opinion.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Claims: Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Harry J. McArdle, Yolanda Sanz, Alfonso Siani, John Joseph Strain, Hendrik Van Loveren and Peter Willatts for the preparatory work on this scientific opinion.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst K-I, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Stern M, Tomé D, Van Loveren H, Vinceti M, Willatts P, Martin A, Strain JJ and Siani A, 2017. Scientific Opinion on 'Nutrimune®' and immune defence against pathogens in the gastrointestinal and upper respiratory tracts: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA Journal 2017;15(1):4679, 14 pp. doi:10.2903/j.efsa.2017.4679

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following an application from H.J. Heinz Supply Chain Europe B.V., submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of the Netherlands, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to 'Nutrimune®' and immune defence against pathogens in the gastrointestinal and upper respiratory tracts.

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

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the EFSA general guidance for stakeholders on health claims applications and the guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms.

The food that is the subject of the health claim is 'Nutrimune®'. The Panel considers that 'Nutrimune®' (a pasteurised cow's skim milk fermented with *Lactobacillus paracasei* CBA L74) is sufficiently characterised.

The claimed effect proposed by the applicant is 'support of the immune defence in the gastrointestinal and upper respiratory tract'. The target population proposed by the applicant is 'young children aged 12–48 months old'. The Panel considers that immune defence against pathogens in the gastrointestinal and upper respiratory tracts is a beneficial physiological effect.

The applicant identified two human intervention studies (one published and one unpublished) which investigated the effects of 'Nutrimune®' on clinical outcomes related infectious diseases of the gastrointestinal (GI) tract and/or upper respiratory tract (URT) in children as being pertinent to the claim.

The Panel notes that one human intervention study from which conclusions could be drawn showed an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT. The Panel also notes, however, inconsistencies in the reporting of the process and criteria used for the diagnosis of upper respiratory tract infections (URTI), and that the results of the study have not been replicated.

The applicant provided one animal study for the scientific substantiation of the claim. The Panel considers that the results from this animal study may be in line with an effect of 'Nutrimune®' on defence against pathogens in the GI tract, albeit the effects shown are small, and found in a model that is very different from a normal infection in humans.

The applicant provided three *in vitro* studies in relation to the mechanism by which 'Nutrimune®' could exert the claimed effect. Based on the information provided, the Panel considers that the results of these studies do not provide evidence for a plausible mechanism by which 'Nutrimune®' could exert the claimed effect *in vivo* in humans.

In weighing the evidence, the Panel took into account that one human intervention study from which conclusions could be drawn showed an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT, and that the results from one animal study could support an effect of 'Nutrimune®' on defence against pathogens in the GI tract. The Panel also took into account the inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI in the human intervention study, that the results of this study have not been replicated, and that no evidence was provided for a plausible mechanism by which 'Nutrimune®' could exert the claimed effect *in vivo* in humans.

On the basis of data presented, the Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of 'Nutrimune®' and immune defence against pathogens in the gastrointestinal and upper respiratory tracts.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006[1] 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 this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14–17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction in disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

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

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) 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: 'Nutrimune®' and immune defence against pathogens.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of 'Nutrimune®', a positive assessment of its safety, nor a decision on whether 'Nutrimune®' 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 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

2.1.1. Information provided by the applicant

2.1.1.1. Food/constituent as stated by the applicant

According to the applicant, the food for which a health claim is made is 'Nutrimune®', which is a heat-treated fermented milk, fermented with *Lactobacillus paracasei* CBA L74.

2.1.1.2. Health relationship as claimed by the applicant

According to the applicant, consumption of 'Nutrimune®' supports the immune defence, thus maintaining immune defence in the gastrointestinal (GI) and upper respiratory tracts, as demonstrated by subsequent reduced occurrence, of both GI and upper respiratory tract infections in combination with a corresponding change in positive stimulation of elements of innate and acquired immunity.

2.1.1.3. Mechanism(s) by which the food exerts the claimed effect as proposed by the applicant

According to the applicant, the mechanism involved in the maintenance of immune defence of the GI and upper respiratory tracts by 'Nutrimune®', may be at least in part related to a positive stimulation of innate and acquired immunity. A negative correlation between faecal concentrations of α- and β-defensins, cathelicidin (LL-37) and secretory immunoglobulin A (sIgA) and the number of infectious episodes has been demonstrated. In a non-human model, a significant positive modulation of non-immune defence mechanisms has been also demonstrated consisting of stimulation of epithelial cells growth, differentiation, permeability and mucus production.

[1] 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. OJ L 404, 30.12.2006, p. 9–25.

2.1.1.4. Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Nutrimune®' supports the immune defence in the gastrointestinal and upper respiratory tract of young children.

2.1.1.5. Specific conditions of use as proposed by the applicant

According to the applicant, doses of 7 g of 'Nutrimune®', in spray-dried form, should have been consumed daily for a period of 3 months to obtain the claimed effect. The given amount of 'Nutrimune®' can be obtained through consumption of a range of products to which 'Nutrimune®' can be added. It is anticipated that 'Nutrimune®' will be applied as an ingredient in liquid, semiliquid and dry forms to a variety of products.

The target population proposed by the applicant are young children aged 12–48 months old.

2.1.2. Data provided by the applicant

Health claim application on 'Nutrimune®' and immune defence against pathogens pursuant to Article 14 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.[2]

As outlined in the General guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a), it is the responsibility of the applicant to provide the totality of the available evidence.

This health claim application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006. Data claimed to be proprietary and confidential by the applicant include:

- Data related to the unpublished human study (Corsello et al., 2015) are proprietary to H.J. Heinz Supply Chain Europe B.V.;
- Data related to the primers sequence presented in the application are confidential to H.J. Heinz Supply Chain Europe B.V.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a).

The scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms are outlined in a specific EFSA guidance (EFSA NDA Panel, 2016b).

3. Assessment

3.1. Characterisation of the food/constituent

The food that is the subject of the health claim is 'Nutrimune®'.

'Nutrimune®' is cow's skim milk fermented with *Lactobacillus paracasei* CBA L74. Fermentation is followed by pasteurisation to kill viable bacteria. The final product is available as a spray-dried milk powder containing at least 7 x 10^{10} colony-forming units (cfu) of non-viable *L. paracasei* CBA L74. It is anticipated that 'Nutrimune®' will be used as an ingredient in liquid, semiliquid and dry forms in a variety of food products.

Lactobacillus paracasei CBA L74 has been deposited in the internationally recognised Belgian collection BCCM/LMG with the International Depository Access Number LMG P-24778.

The species was identified using repetitive extragenic palindromic PCR (rep–PCR) and a specific PCR. A single primer fingerprinting technique was used to identify the strain. Upon a request for clarification from EFSA, the applicant provided results of the 16S rRNA and 23S rRNA gene sequence analysis, confirming the identification of the bacterial strain.

Detailed specifications of the manufacturing process, nutritional composition of the final product, and information on stability, were provided by the applicant.

[2] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific and technical guidance for the preparation and presentation of an application for authorisation of a health claim (revision 1). EFSA Journal 2011;9(5):2170, 36 pp. doi:10.2903/j.efsa.2011.2170

The Panel considers that the food 'Nutrimune®' (a pasteurised cow's skim milk fermented with *Lactobacillus paracasei* CBA L74), which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'support of the immune defence in the gastrointestinal and upper respiratory tract'. The target population proposed by the applicant is 'young children aged 12–48 months old'.

In response to a request for clarification from EFSA, the applicant indicated that the claimed effect relates to 'immune defence against pathogens in the gastrointestinal and upper respiratory tracts'.

As explained in the Guidance on the scientific requirements for health claims related to the immune system, the GI tract and defence against pathogenic microorganisms (EFSA NDA Panel, 2016b), the scientific evidence for the substantiation of health claims related to defence against pathogens in the upper respiratory tract (URT) can be obtained from human intervention studies showing an effect on clinical outcomes related to infections (e.g. incidence, severity and/or duration of symptoms) of the URT (e.g. rhinitis, pharyngitis, sinusitis, otitis media, common cold). Upper respiratory tract infections (URTIs) clinically diagnosed by the primary care or hospital physician following well defined criteria can be used as an appropriate outcome variable for the scientific substantiation of the claim, provided that adequate exclusion criteria for the most common non-infectious causes (e.g. allergic diseases) of the signs and symptoms used for diagnosis of the upper respiratory infection have been applied (i.e. differential diagnosis). Microbiological data could also be used to ascertain the infectious aetiology of clinically diagnosed episodes.

For health claims related to defence against pathogens in the GI tract, clinical outcomes related to GI infections, for example incidence, severity and/or duration of diarrhoeal episodes, could be used. The infectious aetiology of diarrhoeal episodes, however, should be ascertained. In this context, GI infection clinically diagnosed by the primary care or hospital physician following well defined criteria can be used as an appropriate outcome variable for the scientific substantiation of the claim, provided that adequate exclusion criteria for the most common non-infectious causes of diarrhoea have been applied. Microbiological data could also be used to ascertain the infectious aetiology of diarrhoeal episodes.

Other outcome variables, such as changes in relevant immunological markers, may provide supportive evidence on the mechanism (e.g. through the activation of the immune system) by which the food/constituent could exert the claimed effect, but alone are not appropriate for the substantiation of claims related to immune defence against pathogens.

The Panel considers that immune defence against pathogens in the GI and upper respiratory tracts is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in the Cochrane library, PubMed and Clinical Trials databases using the following key words: *Lactobacillus paracasei* and CBA L74, immune defence against pathogens and/or clinical outcomes of infections, and human and/or *in vivo* data. No limits were set. Additionally, hand searching was used to identify publications on *Lactobacillus paracasei* CBA L74.

As a result, the applicant identified two human intervention studies (one published and one unpublished) which investigated the effects of 'Nutrimune®' on clinical outcomes related infectious diseases of the GI and/or respiratory tracts in children as being pertinent to the claim. The applicant also provided one animal efficacy study and three *in vitro* studies in relation to the mechanism by which 'Nutrimune®' could exert the claimed effect.

3.3.1. Human intervention (efficacy) studies

In a randomised, double-blind, one-centre (performed in paediatric centre in Naples), three-arm parallel study, Nocerino et al. (2015) evaluated the effect of 'Nutrimune®' and of rice fermented with *Lactobacillus paracasei* CBA L74 compared with placebo on the incidence of common infectious diseases (CID) in healthy young children aged 12–48 months who attended day care or preschool at least 5 days per week.

The exclusion criteria were: concomitant chronic systemic diseases, congenital cardiac defects, gastrointestinal or urinary or respiratory tract surgery, active tuberculosis, autoimmune diseases, immunodeficiency, chronic inflammatory bowel diseases, cystic fibrosis, metabolic diseases, history of suspected or challenge-proved food allergy, lactose intolerance, malignancy, chronic pulmonary diseases, malformations of gastrointestinal or urinary or respiratory tract, severe malnutrition, and use of 'pre/pro/synbiotics', antibiotics or immune stimulating products in the 2 weeks before the enrolment.

A sample size of 118 children per group was calculated for a power of 0.92 and $\alpha = 0.05$, assuming the occurrence of at least one episode of infection in 80% of children in the placebo group and at least one episode of infection in 60% of children in both intervention groups. Randomisation was performed with a computer-generated randomisation sequence using blocks of 36 subjects per family paediatrician. The investigators were blinded in relation to the allocation of the participants, the intervention, laboratory analysis and statistical analysis.

A total of 432 children (51% male, mean age 32 months, n = 144 per group) were randomised to receive daily either 'Nutrimune®', rice fermented with *Lactobacillus paracasei* CBA L74, or placebo (maltodextrin) for 3 months (January–March 2012). All tested products were given as a powder (7 g/day) to be diluted in a maximum of 150 mL of cow's milk or water. Parents were instructed to exclude 'prebiotics, probiotics, synbiotics and immune stimulating products' during the intervention period. Compliance was defined as the consumption of at least 80% of the assigned treatment during the study.

A number of participants refused to participate after randomisation (three in the 'Nutrimune®' group, 21 in the fermented rice group and 17 in the placebo group). Upon a request for clarification from EFSA, the applicant explained that the reasons for refusal were change in residence (n = 5), withdrawal of informed consent (n = 12) and the use of 'pre/pro/synbiotics or antibiotics' after randomisation but before treatment (n = 24). The Panel notes that the latter reason for withdrawal was unequally distributed among the study groups (one in the 'Nutrimune®', 12 in the fermented rice, and 11 in the placebo group).

The primary outcome of the study was the proportion of children experiencing at least one episode of CID affecting the respiratory or the GI tracts. The secondary outcomes included the proportion of children with recurrent CID (i.e. ≥ 3 episodes), the total number of CID's, the use of medications (antipyretics, antibiotics or steroids), number of visits to the emergency department, number of paediatric visits and number of hospitalisations. Markers of innate (α- and β defensins, and cathelicidin LL-37) and acquired (secretory immunoglobulin A) immunity were measured in faeces at baseline and at the end of the intervention.

A diary was filled in by the parents recording fever, GI and respiratory symptoms, use of medications, visits to the emergency department, hospitalisations, and possible adverse events. There were three planned visits with the family paediatricians (at 30, 60 and 90 days). Additional visits took place whenever it was necessary because of the suspicion of an infectious disease or other morbidities. The diagnosis of infectious diseases was made by paediatricians based on the evaluation of specific symptoms according to standardised definitions.

Acute gastroenteritis was suspected by the presence of ≥ 3 episodes of soft/liquid faeces in 24 h with or without fever or vomiting. The presence of URTI was defined as the occurrence of ≥ 1 respiratory symptom(s) (runny nose, cough, sore throat, aphony, shortness of breath, otalgia, otorrhoea, extroversion of tympanic membrane with or without hyperaemia) in the absence or presence of ≥ 1 systemic symptoms(s) (fever, headache, restless, myalgia, irritability). The applicant stated that the same definition of URTI was used in several previous studies (Hojsak et al., 2010; Agustina et al., 2012; Maldonado et al., 2012). Upon a request from EFSA for clarification on whether one of the symptoms listed above was sufficient for the diagnosis of an URTI, the applicant explained that all medical examinations were performed by the same family paediatrician for each child throughout the whole study period and that the diary compiled by the parents served only to flag potential symptoms of infections and to trigger a visit with the family paediatrician. At these visits, the paediatrician inspected the diary completed by the parents, performed a full physical examination and then, using standard definitions based on clinical practice guidelines issued by national and

international scientific societies,[3] decided on the diagnosis. In a further request for clarification by EFSA, the applicant specified that the acute occurrence of at least one symptom suggestive of rhinitis, tracheitis, laryngitis, pharyngitis or acute otitis media, as reported in the diary, was mandatory for the final diagnosis of URTI, although a cluster of signs and symptoms were evaluated by the paediatrician in line with the guidelines in all cases to make the final diagnosis. Microbiological investigations were not performed and the objective signs of cold (e.g. nasal mucus weight, nasal mucociliary clearance function) were not used for diagnosis.

In response to a request for clarification by EFSA on the criteria used to exclude the most common non-infectious causes of the signs and symptoms used for the diagnosis of URTI and GI infections (i.e. differential diagnosis), the applicant confirmed that children with antibiotic-associated diarrhoea and children with diagnosis of allergic diseases were excluded from the study. In addition, the applicant stated that possible newly developed allergies could have been detected by family paediatricians.

The Panel notes that the process and the criteria used for the diagnosis of infections reported in the publication (Nocerino et al., 2015) and in the study report provided with the initial submission of the application significantly differ from those described in subsequent clarifications obtained from the applicant during scientific evaluation of the claim. In this context, the Panel assumes that the diagnosis of URTI was made by physicians based on a medical history and a physical examination according with accepted diagnostic standards, which are based on clusters of symptoms, as specified by the applicant in its last clarification,[3] and that at least one acute symptom suggestive of rhinitis, tracheitis, laryngitis, pharyngitis or acute otitis media was needed (but not sufficient) for the diagnosis. The Panel also notes the exclusion of children with diagnosis of allergic diseases and/or under antibiotic treatment to exclude the most common non-infectious causes of the signs and symptoms used for the diagnosis of URTI and GI infections.

The vaccination status was identical among all studies groups. No child had received anti-rotavirus or anti-influenza vaccine.

Fourteen participants dropped out during the study (lost to follow-up): four in the 'Nutrimune®', five in the fermented rice and five in the placebo group. The 'Intention to Treat (ITT)' analysis was performed in children who received at least one dose of the allocated intervention, and did not include children who refused to participate after randomisation. The Panel notes that the 'ITT' analysis was a full analysis set (FAS).

The main outcome of the study (i.e. the proportion of children experiencing at least one episode of CID affecting the respiratory or the GI tracts) was assessed using FAS analysis, assuming that all children with missing data (n = 14) had one CID. Binomial regression was used to calculate the absolute risk difference for the occurrence of at least one CID in both intervention groups against the placebo group.

Per protocol (PP) analyses were performed separately for acute URTI and acute GI infections. The incidence rate ratio of CID in both intervention groups against the placebo group was estimated using the Poisson regression analysis. Statistical analyses of secondary outcomes were performed in the PP population only.

Changes in α-defensin, β-defensin, LL-37 and sIgA were analysed using a random effect linear regression. The treatment $\times$ time interaction was calculated to assess changes in the immunological markers for the intervention groups vs placebo at 3 months vs baseline. Immunological markers were

[3] Rhinitis was defined by the acute onset of rhinorrhoea (mucus secretion from the nose), nasal airways obstruction with or without cough, sneezing, fever and conjunctivitis (Kliegman et al., 2009; De Martino, 2012; Principi et al., 2012).
Tracheitis was defined by acute onset of signs and/or symptoms of airway obstruction or impending respiratory failure or both. These symptoms may include tachypnoea, cough, mucus production or fever (Kliegman et al., 2009; Principi et al., 2012).
Laryngitis was defined by the acute onset of inspiratory wheezing with cough and hoarse voice with or without chest indrawing and stridor (Kliegman et al., 2009; De Martino, 2012; Principi et al., 2012).
Pharyngitis (or sore throat) was defined by the acute onset of inflammation of the pharyngeal tonsils that may be accompanied by other nonspecific symptoms, including cough, pharyngodynia and fever (Kliegman et al., 2009; De Martino, 2012).
Acute otitis media was defined by the acute onset of symptoms (otalgia, otorrhoea, irritability, fever) and the result of a careful otoscopic examination to confirm the presence of inflammatory changes in the lymphatic membrane. These signs include bulging with or without erythema and one or more of the acute symptoms. For the otoscopic examination, this entailed the demonstration of tympanic membrane inflammation based on: (a) otoscopic findings of marked erythema of the tympanic membrane, with bulging and the absence mobility due to the presence of middle ear effusion or (b) otoscopic findings of a yellowish membrane by observing in transparency the presence of purulent material in the middle ear or (c) the presence of spontaneous perforation with otorrhoea. Redness alone of the tympanic membrane was considered insufficient for the diagnosis (Kliegman et al., 2009; Marchisio et al., 2010; De Martino, 2012). In order to help the family paediatricians in distinguishing acute otitis media from otitis media with effusion, a video provided by the American Association of Paeditricians was used during the instructions provided at the two investigator meetings.

log_e-transformed (skewed distribution) to ensure homoscedasticity of residuals. Statistical analysis was performed using SPSS 19.0 for Windows and Stata 14.1.

The statistical analysis was performed separately for each intervention group against the placebo group.

The Panel considers that the results obtained in the fermented rice group are not relevant for the scientific substantiation of a claim on 'Nutrimune®', the food that is the subject of this application, and therefore are omitted in this opinion.

In the FAS analysis, the number of children with at least one CID was significantly lower in the 'Nutrimune®' group (n = 73) than in the placebo group (n = 102; $p < 0.0001$). The absolute risk difference in the occurrence of at least one CID was −29% (95% CI: −39% to −18%, $p < 0.001$) for the 'Nutrimune®' group vs. placebo. The total number of CIDs was 129 in the 'Nutrimune®' group and 324 in the placebo group. No adverse events were noted in any of the groups.

In the PP analysis (children complying with the protocol), the proportion of children presenting at least one episode of acute gastroenteritis was significantly lower in the 'Nutrimune®' than in the placebo group. The absolute risk difference in the occurrence of at least one episode of acute gastroenteritis was −18% (95% CI: −28% to −8%, $p < 0.001$) for the 'Nutrimune®' group vs placebo. The absolute risk difference in the occurrence of at least one episode of URTI was −22% (95% CI: −34% to −11%, $p = 0.001$) in the 'Nutrimune®' group vs placebo. The Incidence Rate Ratio (IRR) calculated using Poisson regression was 0.36 (95% CI: 0.29–0.44, $p < 0.001$) in the 'Nutrimune®' group vs placebo.

The proportion of subjects who experienced at least one episode of acute gastroenteritis (n = 18% in 'Nutrimune®' vs 38% in placebo, $p < 0.0001$), rhinitis (n = 19% in 'Nutrimune®' vs 35% in placebo, $p = 0.003$), otitis (n = 3% in 'Nutrimune®' vs. 18% in placebo, $p < 0.0001$), pharyngitis (n = 21% in 'Nutrimune®' vs 53% in placebo, $p < 0.0001$), laryngitis (n = 9% in 'Nutrimune®' vs 22% in placebo, $p = 0.005$) and tracheitis (n = 36% in 'Nutrimune®' vs 49% in group, $p < 0.018$) was significantly lower in the 'Nutrimune®' group compared to placebo. Differences remained statistically significant (except for tracheitis) after Bonferroni correction. It was reported that the total number of episodes of the individual URTIs was also significantly lower in the 'Nutrimune®' group compared to placebo.

The odds ratio (OR) of receiving at least one medication were significantly lower in the 'Nutrimune®' group compared to placebo (OR 0.26, 95% CI: 0.15–0.43). The number of children taking at least one medication during the intervention was 41 in the 'Nutrimune®' group and 76 in the placebo group. The additional number of paediatric visits was 142 in the 'Nutrimune®' group and 353 in the placebo group. Emergency visits or hospitalisations were not required.

The number of children with ≥ 3 CID episodes was lower in the 'Nutrimune®' group (n = 14) than in the placebo group (n = 57, $p < 0.001$). The proportion of children with recurrent CID (i.e. ≥ 3) vs those with one or two infectious episodes was 10% in the 'Nutrimune®' group and 37% in the placebo group ($p < 0.001$ in Wald test, binomial regression).

Faecal α-defensin, β-defensin, LL-37 and sIgA significantly increased in the 'Nutrimune®' group as compared to placebo ($p < 0.001$ for all comparisons at 3 months vs baseline). An inverse association was also reported between changes in α-defensin ($\rho = -0.362$, $p = 0.004$), β-defensin ($\rho = -0.256$, $p = 0.047$), LL-37 ($\rho = -0.296$, $p = 0.012$) and sIgA ($\rho = -0.356$, $p = 0.001$) and the total number of CIDs at the end of the intervention.

The Panel considers that this study shows an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT. The Panel notes, however, inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI in the study.

The second human intervention study submitted (Corsello et al., 2015, unpublished, claimed as proprietary by the applicant) was a randomised, parallel, double-blind, placebo-controlled, two-arm study. The study was conducted in three Italian paediatric centres (i.e. Naples, Palermo and Milan) and investigated the effect of 'Nutrimune®' on the incidence of CID in a group of healthy children attending day care or preschool at least 5 days a week.

Except for the absence of a third study arm receiving fermented rice, the study protocol (inclusion and exclusion criteria for the selection of subjects, study products and doses used, duration of the intervention, assessment of compliance, vaccination status, primary and secondary outcomes, and statistical analysis) was as described for the previous intervention study (Nocerino et al., 2015). In addition, the number of days lost from school and the number of working days lost by parents were assessed. The study was conducted between December 2014 and March 2015.

It was calculated that 73 children per group were needed for a power of 0.90 at $\alpha = 0.05$ assuming the occurrence of at least one episode of infection in 79% of the children in the placebo group and in 50% of the children in intervention group.

A total of 146 children were randomised (using a computer-generated randomisation sequence) to receive either 'Nutrimune®' (n = 73, 53% male, mean age 39 months) or placebo (n = 73, 62% male, mean age 45 months) for 3 months. All randomised children received the allocated intervention.

Twenty children dropped out during the study (lost to follow-up): seven in the 'Nutrimune®' and 13 in the placebo group. The ITT analysis on the primary outcome was conducted in the 146 children randomised, assuming that all children lost to follow-up had one episode of CID. The PP analysis was performed in the 126 children who completed the study and complied with the protocol as planned (66 in the 'Nutrimune®' and 60 in the placebo group).

The results of 'pooled' ITT and PP analyses for the primary and secondary outcome variables were provided. However, the Panel noted that the multicentre design had not been considered in data analysis.

Following a request for clarification by EFSA, the applicant acknowledged that the study was designed to detect a given effect size on the primary outcome on the 'pooled' sample of children recruited, and thus the potential role of study centre had to be taken into account in secondary analyses. In this context, the applicant provided the results of a binomial regression model using treatment and centre (0 = Naples; 1 = Milan and 2 = Palermo) as discrete variables.

The Panel notes that the vast majority of the participants were recruited in one centre (i.e. 105 in Naples, 17 in Milan and 24 in Palermo), that the statistical analysis provided is not appropriate for the study data (i.e. the same weight is given to all centres, regardless of their sample size), and that if the study was not planned, designed, randomised and analysed as a multicentre study (as acknowledged by the applicant), the aleatory recruitment of subjects in three different centres is not duly justified. The Panel also notes that this study shares the inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI identified for the study by Nocerino et al. (2015).

The Panel considers that this study is at high risk of bias and no conclusions can be drawn for the scientific substantiation of the claim.

The Panel notes that one human intervention study from which conclusions could be drawn showed an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT. The Panel also notes, however, inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI, and that the results of the study have not been replicated.

3.3.2. Animal efficacy studies

The applicant also provided one animal study for the scientific substantiation of the claim (Zagato et al., 2014). Twenty mice were fed with 'Nutrimune®' or non-fermented milk (control) for 10 days and then were challenged intragastrically with a lethal dose of *Salmonella* Typhimurium FB62 (10^6 cfu in 200 μL carbonate buffer). Mice receiving 'Nutrimune®' survived slightly, but statistically significantly, longer than mice in the control group.

The Panel considers that the results from this animal study may be in line with an effect of 'Nutrimune®' on defence against pathogens in the GI tract, albeit the effects shown are small, and found in a model that is very different from a normal infection in humans.

3.3.3. Mechanism of action

The applicant claims that the observed negative correlation between increased faecal concentrations of α- and β-defensins, cathelicidin (LL-37) and secretory IgA (sIgA) and the number of infectious episodes in human intervention study (Nocerino et al., 2015), indicates that the biological mechanism behind this effect may be at least in part *via* a positive stimulation of innate and acquired immunity. 'Nutrimune®' significantly increased faecal concentrations of α- and β-defensins, LL-37 and sIgA in the human intervention study provided (Nocerino et al., 2015), and it was speculated that the ingredient reaches the gut in active form to enable direct effects on intestinal cells (both epithelial and immune cells) and may explain the reduced risk for GI infections.

As for the URTI, the applicant argues that 'the immune modulatory effect of 'Nutrimune®' may be transmitted to immune components (e.g. T and B lymphocytes) able to reach other mucosa-associated lymphoid tissues, e.g. in the respiratory tract', and that 'IgA-producing B cells generated at intestinal sites may migrate to other mucosal sites to offer protection against invading pathogens'. However, no evidence was provided by the applicant to support this statement.

The applicant also states that two references submitted as abstracts (Sarno et al., 2014; Paparo et al., 2015) could provide evidence for a non-immune defence mechanism by which 'Nutrimune®' could provide defence against pathogens in the GI tract (i.e. 'by stimulation of epithelial cells growth, differentiation, permeability and mucus production through a direct interaction with the enterocytes'). The Panel notes that the information provided in these two abstracts is not sufficient to allow a full scientific evaluation.

Finally, the applicant claims that 'Nutrimune®' showed 'strong anti-inflammatory' effects *in vitro* by reducing IL-12p70 production while preserving the production of IL-10 by human monocyte-derived dendritic cells stimulated with *Salmonella* Typhimurium; *ex vivo,* by reducing the capacity of *Salmonella* Typhimurium to cause inflammation and tissue destruction in colon explants; and *in vivo*, by its protective effects against dextran sulfate sodium (DSS)-induced colitis in mice (Zagato et al., 2014). The Panel notes that although an anti-inflammatory response is part of the physiological response to a pro-inflammatory stimulus (such as an infectious agent), no evidence has been provided that this is part of the mechanism by which 'Nutrimune®' exerted an effect in humans. The Panel also notes that the animal model used in the second study provided (i.e. the DSS-colitis murine model) is not appropriate to substantiate a claim on infections.

The Panel considers that the results of the studies discussed in this section do not provide evidence for a plausible mechanism by which 'Nutrimune®' could exert the claimed effect *in vivo* in humans.

3.3.4. Weighing of the evidence

In weighing the evidence, the Panel took into account that one human intervention study from which conclusions could be drawn showed an effect of 'Nutrimune®' on immune defence against pathogens in the GI tract and the URT, and that the results from one animal study could support an effect of 'Nutrimune®' on defence against pathogens in the GI tract. The Panel also took into account the inconsistencies in the reporting of the process and criteria used for the diagnosis of URTI in the human intervention study, that the results of this study have not been replicated, and that no evidence was provided for a plausible mechanism by which 'Nutrimune®' could exert the claimed effect *in vivo* in humans.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of 'Nutrimune®' and immune defence against pathogens in the GI and upper respiratory tracts.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food constituent 'Nutrimune®' (a pasteurised cow's skim milk fermented with *Lactobacillus paracasei* CBA L74), which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'support of the immune defence in the gastrointestinal and upper respiratory tract'. The target population proposed by the applicant is young children aged 12–48 months. Immune defence against pathogens in the GI and upper respiratory tracts is a beneficial physiological effect.
- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of 'Nutrimune®' and immune defence against pathogens in the GI and upper respiratory tracts.

Documentation provided to EFSA

1) Health claim application on 'Nutrimune®' and 'immune defence against pathogens' pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0445_NL). Submitted by H.J. Heinz Supply Chain Europe B.V., Nieuwe Dukenburgseweg 19, Nieuwe Dukenburgseweg 19, The Netherlands.
2) This application was received by EFSA on 05/1/2016.
3) The scope of the application was proposed to fall under a health claim referring to disease risk reduction.
4) On 28/1/2016, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
5) On 20/2/2016, EFSA received the missing information as submitted by the applicant.
6) The scientific evaluation procedure started on 4/3/2016.

7) On 17/3/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was stopped on 28/ 4/2016 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
8) On 13/5/2016, EFSA received the requested information and the scientific evaluation was restarted.
9) On 9/6/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was stopped on 8/7/2016 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
10) On 26/7/2016, EFSA received the requested information and the scientific evaluation was restarted.
11) On 6/9/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was stopped on 3/10/2016 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
12) On 10/11/2016, EFSA received the requested information and the scientific evaluation was restarted.
13) During its meeting on 14/12/2016, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to 'Nutrimune®' and immune defence against pathogens.

References

Agustina R, Kok FJ, van de Rest O, Fahmida U, Firmansyah A, Lukito W, Feskens EJM, van den Heuvel EGHM, Albers R and Bovee-Oudenhoven IMJ, 2012. Randomized trial of probiotics and calcium on diarrhea and respiratory tract infections in Indonesian children. Pediatrics, 129, 1155–1164.

Corsello G, Berni Canani R and Mosca F, 2015 Effect of fermented milk with *Lactobacillus paracasei* CBA L74 on gastrointestinal and respiratory infections in children: a multicenter randomized controlled trial. Unpublished study report.

De Martino M, 2012. *Pediatria*. Edises, Napoli.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016a. General scientific guidance for stakeholders on health claim applications. EFSA Journal 2016;14(1):4367, 38 pp. doi:10.2903/j.efsa.2016.4367

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016b. Guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms. EFSA Journal 2016;14(1):4369, 23 pp. doi:10.2903/j.efsa.2016.4369

Hojsak I, Abdovic S, Szajewska H, Milosevic M, Krznaric Z and Kolacek S, 2010. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. Pediatrics, 125, 1171–1177.

Kliegman R, Behrman R, Jenson H and Stanton B, 2009. *Nelson textbook of pediatrics*, 18th edition. Saunders, Philadelphia.

Maldonado J, Canabate F, Sempere L, Vela F, Sanchez AR, Narbona E, Lopez-Huertas E, Geerlings A, Valero AD, Olivares M and Lara-Villoslada F, 2012. Human milk probiotic Lactobacillus fermentum CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. Journal of Pediatric Gastroenterology and Nutrition, 54, 55–61.

Marchisio P, Bellussi L, Di Mauro G and Principi N, 2010. Acute otitis media: from diagnosis to prevention. Summary of the Italian guideline. International Journal of Pediatric Otorhinolaryngology, 74, 1209–1216.

Nocerino R, Paparo L, Terrin G, Pezzella V, Amoroso A and Cosenza L, 2015. Cow's milk and fermented rice with *Lactobacillus paracasei* CBA L74 prevent infectious diseases in children: a randomized controlled trial. Clinical Nutrition. doi: 2015.12.004 (in press).

Paparo L, Aitoro R, Nocerino R, Maddalena Y, Pezzella V, Amoroso A, Di Scala C, Siciiano E, Buono B and Berni Canani R, 2015. Direct effects of fermented cow's milk with *Lactobacillus paracasei* CBA L74 on human enterocytes. Journal of Pediatric Gastroenterology and Nutrition, 60(Suppl. 1), 732 (abstract).

Principi N, Rubino A and Vierucci A, 2012. *Pediatria generale e specialistica*, 2nd edition. Casa Editrice Ambrosiana, Milano.

Sarno M, Buccigrossi V, Nanayakkara M, Lania G, Kosova R, Cuomo M, Russo C, Troncone R, Guarino A and Barone MV, 2014. *Lactobacillus paracasei* CBA L74 prevents entrance of undigested gliadin peptides and Rotavirus in Caco-2 cells. ESPGHAN abstract book, 184 (abstract).

Zagato E, Mileti E, Massimiliano L, Fasano F, Budelli A, Penna G and Rescigno M, 2014. *Lactobacillus paracasei* CBA L74 metabolic products and fermented milk for infant formula have anti-inflammatory activity on dendritic cells in vitro and protective effects against colitis and an enteric pathogen in vivo. PLoS ONE, 9, e87615.

Abbreviations

cfu	colony-forming units
CID	Common Infectious Disease
DSS	dextran sulfate sodium
FAS	Full Analysis Set
GI	gastrointestinal
IRR	Incidence Rate Ratio
ITT	Intention to Treat
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
PCR	polymerase Chain Reaction
PP	per protocol
RNA	ribonucleic acid
URT	upper respiratory tract
URTI	upper respiratory tract infection