SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to fermented milk containing *Lactobacillus casei* DN-114 001 plus yoghurt symbiosis (Actimel®), and reduction of *Clostridium difficile* toxins in the gut of patients receiving antibiotics and reduced risk of acute diarrhoea in patients receiving antibiotics pursuant to Article 14 of Regulation (EC) No 1924/2006[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Danone Produits Frais France submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to a fermented milk drink Actimel® containing *Lactobacillus casei (Lc)* DN-114 001 and reduction of the presence of *Clostridium difficile* toxins in the gut which reduces the incidence of acute diarrhoea. The Panel considers that the food constituent, Actimel®, which is the subject of the health claim, is sufficiently characterised. The Panel considers that reducing the risk of *Clostridium difficile* diarrhoea by reducing the presence of *C. difficile* toxins is a beneficial physiological effect. In total the applicant indicated seven publications on human studies, three unpublished human studies, eight published and one unpublished non-human studies to be pertinent for the claimed effect. In weighing the evidence, the Panel took into account that human and animal studies showed partial survival of *Lc* DN-114 001 during its gastrointestinal passage, that one human intervention study with Actimel® which showed a statistically significant risk reduction for CDAD had considerable limitations, that there were only limited data on the effect of Actimel® on the reduction *C. difficile* toxins (the risk factor) in humans, that one study which showed an inhibitory effect of *Lc* DN-114 001 on the growth of *C. difficile in vitro* does not predict the occurrence of an effect against *C. difficile* in humans, that five further human studies do not support the proposed mechanisms by which Actimel® could exert the claimed effect, and that the evidence provided from a further two

1 On request from the Competent Authority of France following an application from Danone Produits Frais France, Question No EFSA-Q-2009-00776, adopted on 12 November 2010.

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3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: 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 and Hans Verhagen for the preparatory work on this scientific opinion and EFSA staff: Wolfgang Gelbmann for the support provided to this scientific opinion.

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animal and three *in vitro* studies does not establish that effects of Actimel® or *Lc* DN-114 001 in these model systems related to immune function and infection can predict the occurrence of such effects in humans. The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of Actimel® and a reduction of the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins.

KEY WORDS

Actimel®, Lactobacillus casei DN-114 001, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, yoghurt, fermented milk, microbiota, intestine, Clostridium difficile toxin, risk reduction, elderly, diarrhoea, antibiotic treatment.

SUMMARY

Following an application from Danone Produits Frais France submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to a fermented milk drink containing *Lactobacillus casei* DN-114 001 plus yoghurt symbiosis (Actimel®) and reduction of the presence of *Clostridium difficile (C. difficile)* toxins in the gut which reduces the incidence of acute diarrhoea.

The scope of the application was proposed to fall under a health claim referring to disease risk reduction.

The food constituent that is the subject of the health claim is a fermented milk product (Actimel®) containing *Lactobacillus casei* DN-114001 and yoghurt cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). The identification and characterisation of the strains have been performed by applying phenotypic and genotypic methods. The Panel considers that the food constituent, Actimel®, which is the subject of the health claim, is sufficiently characterised.

The claimed effect is "decreases the presence of *C. difficile* toxins (the risk factor), in the intestinal tract and reduces the incidence of acute diarrhoea associated with their presence in the gut of susceptible ageing people". The target population is adults over 50 years old receiving antibiotic treatment. The Panel considers that reducing the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins is a beneficial physiological effect.

In total the applicant indicated seven publications on human studies, three unpublished human studies, eight published and one unpublished non-human studies to be pertinent for the claimed effect. In addition the applicant provided 65 references related to *C. difficile*, antibiotic associated diarrhoea (AAD), the homeostasis of the intestine and possible mechanisms of "probiotics". These 65 references did not refer to Actimel® or its bacterial strains. Three human studies referred to *C. difficile* associated diarrhoea (CDAD).

In an open non-controlled observational study with 213 elderly patients with a mean age of 88 years in two geriatric care wards of a hospital, subjects received twice daily Actimel® for the duration of an antibiotic treatment course and 7 days after. The study recorded the incidence of CDAD among patients in the geriatric wards for the period November 2007 to January 2008 and compared it with the historical incidence in the period of November 2006 to January 2007 when this yoghurt drink had not been consumed. The Panel notes that this study was not controlled for factors other than the consumption of Actimel® that might have influenced the incidence of CDAD. The Panel considers that no conclusion can be drawn from this study for the scientific substantiation of the claimed effect.

A randomised, double-blind, placebo-controlled pilot (RCT) study was designed to test the feasibility of a daily intervention with 2 x 100 ml per day of Actimel® and of a non-fermented acidified dairy

drink to investigate the effect on the occurrence of AAD and of CDAD in elderly hospitalised patients receiving antibiotics (10 per group). The applicant indicated in the study report that "no statistical comparison was performed in the context of a small pilot study". The Panel agrees that this pilot study was underpowered to assess the effects of Actimel® on the incidence of AAD and CDAD. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claimed effect.

A RCT with 135 patients of 50 or more years of age under antibiotic treatment studied the effect of daily 2 x 100 ml of the yoghurt drink Actimel® compared to 2 x 100 ml daily of a control product on the occurrence of AAD as the primary endpoint and CDAD as a secondary endpoint. Test and control product were to be consumed twice a day during the course of antibiotic treatment and for one week thereafter. As the products were packaged in bottles of different size (100 g Actimel® bottle versus 200 ml Yazoo® bottle), the hospital pharmacies removed the commercial labels and nursing staff were instructed to pour 100 ml of the respective products into a cup and to deliver to the patients in order to ensure blinding. According to the study protocol, patients who were discharged from the hospital before the end of the intervention were given adequate supplies of test and control products with instructions for use labelled on the bottles. The Panel considers that while the blinding procedure used in the hospital was adequate, the procedure used following discharge could have resulted in un-blinding of patients due to the different size of the bottles for the two products.

"Diarrhoea" was defined as more than two liquid stools a day for three or more days in quantities in excess of normal for each patient. According to the study protocol, stool samples of all patients should have been tested at baseline, when diarrhoea was reported and at the end of the trial. The follow-up of 12 subjects of the Actimel® group and of 10 subjects of the control group could not be completed. In a complete- case analysis (per-protocol analysis), the authors reported AAD for 7 out of 57 patients in the Actimel® group and for 19 out of 56 patients of the control group. In none of the patients in the Actimel® group and in 9 out of 53 of the control group diarrhoea was associated with a stool sample positive for *C. difficile* toxins. This corresponds to a statistically significant 22 % (95 % CI: 7-37) and 17 % (95 % CI: 7-27) absolute risk reduction for the occurrence of AAD and CDAD, respectively. The Panel notes that *C. difficile* toxins were only measured in patients who developed AAD and that there were no data on the effect of Actimel® on the occurrence of *C. difficile* toxins in the faeces of patients without AAD. On request by EFSA, the applicant provided a re-assessment of the data with and without data imputation for the non-completers. The applicant provided a re-assessment with data imputation assuming that a) none of the 22 drop outs had AAD and b) that 20 % in both groups had AAD. The results remained statistically significant ($p < 0.05$) in favour of the test product for both scenarios. The Panel notes that statistical significance is sensitive to the treatment of the missing data and that no data imputation has been applied for more conservative scenarios than 20 % assumed occurrence of AAD.

The Panel notes a number of weaknesses in this study: potential bias through un-blinding of the patients to the products; the lack of data on the presence of *C. difficile* toxins (the risk factor) in the faeces of patients at the end of the intervention; no scenario has been applied for the missing data imputation for more conservative scenarios than 20 % assumed occurrence of AAD. The Panel considers that the weaknesses of this study limit its value as a source of data to substantiate the claim.

An *in vitro* study was provided on the capability of *Lc* DN-114 001 to inhibit the growth of *C. difficile*. According to the summary report the supernatant of *Lc* DN-114 001 was tested with the "overlay method" modified by Danone, for its capacity to inhibit the *in vitro* growth of *Salmonella thyphimurium, Listeria monocytogenes* and *C. difficile*. According to the data provided, the supernatant of *Lc* DN-114 001 tested positive for its capability to inhibit the growth of all three pathogens. The Panel considers that the results of this *in vitro* study do not predict the occurrence of such an inhibitory effect in humans.

Five human studies investigated the kinetics, metabolism and survival of *Lc* DN-114 001 and its impact on the human microbiota during its gastrointestinal transit. These studies demonstrated that *Lc* DN-114 001 can partially survive the gastrointestinal transit. A study demonstrated the expression of 10 genes of *Lc* DN-114 001 in human faeces after gastrointestinal transit and did not find evidence for multiplication. Results from animal studies showed that *Lc* DN-114 001 can partially survive gastrointestinal transit in human flora-associated mice and rats and is excreted without detectable multiplication with the same kinetics as the inert transit marker *Bacillus subtilis* spores. The Panel notes that human and animal data consistently showed partial survival of *Lc* DN-114 001 during its gastrointestinal passage without detectable multiplication.

Regarding possible mechanisms by which Actimel® could reduce the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins, the applicant suggested that Actimel® increased colonisation resistance against pathogens and inhibited pathogen proliferation by modulation of the intestinal microbiota, reduction of the gut epithelial permeability, modulation of gut epithelial receptors and induction of specific IgA production.

The applicant provided two human studies on the effect of Actimel® on gastrointestinal infections and gastrointestinal pathogens, but which did not refer to *C. difficile*. In a RCT with 1072 elderly volunteers consuming 2 x 100 ml Actimel® per day over 3 months there was no difference in the incidence of common infectious diseases, the incidence and duration of gastrointestinal infections, the occurrence of fever, the occurrence of pathogens, the number of prescribed medication and immunological parameters. In another RCT, 250 young adult volunteers enrolled in firefighting training consumed 2 x 100 ml Actimel® per day over 7 weeks. There was no difference in the incidence of common infectious diseases, the incidence of gastrointestinal infections, the occurrence of fever, the occurrence of pathogens in faeces and the number of prescribed medication. The Panel notes that no information has been provided on *C. difficile.* The Panel considers that these two RCTs do not support an effect of Actimel® on the reduction of gastrointestinal infections or gastrointestinal pathogens in humans.

Three other human studies addressed effects on the intestinal microbiota. In an uncontrolled human trial with 12 healthy subjects no statistically significant change occurred after 10 days of daily consumption of 300 ml Actimel® in either the dominant members of the faecal microbiota, bacterial enzyme activities, pH and metabolites such as short-chain fatty acids. This was confirmed by another human trial in 7 volunteers after 8 days of daily consumption of 300 ml Actimel® which did not find any statistically significant change of the proportions of 7 phylogenetic groups . Also a study on the effect of a daily intake of 125 g Actimel® in 39 infants and young children of 10 -18 months of age, did not find any statistically significant modification of the number of total anaerobes, bifidobacteria, bacteroides, and enterobacteria, bacterial metabolites and bacterial enzyme activities in faeces at the end of a 30-day intervention. Based on the parameters measured, the Panel considers that the three human studies do not provide evidence that Actimel® can modulate the human gut microbiota.

The applicant provided another two animal studies and three *in vitro* studies on possible effects of Actimel® or *Lc* DN-114 001 in model systems related to immune function and infection. The Panel notes that none of these five studies investigated *C. difficile*. The Panel considers that the evidence provided does not establish that effects of Actimel® or *Lc* DN-114 001 observed in these animal and *in vitro* studies can predict the occurrence of effects on immune function or infection in humans.

In weighing the evidence, the Panel took into account that human and animal studies showed partial survival of *Lc* DN-114 001 during its gastrointestinal passage, that one human intervention study with Actimel® which showed a statistically significant risk reduction for CDAD had considerable limitations, that there were only limited data on the effect of Actimel® on the reduction *C. difficile* toxins (the risk factor) in humans, that one study which showed an inhibitory effect of *Lc* DN-114 001 on the growth of *C. difficile in vitro* does not predict the occurrence of an effect against *C. difficile* in humans, that five further human studies do not support the proposed mechanisms by which Actimel®

could exert the claimed effect, and that the evidence provided from a further two animal and three *in vitro* studies does not establish that effects of Actimel® or *Lc* DN-114 001 in these model systems related to immune function and infection can predict the occurrence of such effects in humans.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of Actimel® and reduction of the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins.

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 6
Background as provided by the European Commission 7
Terms of reference as provided by the European Commission 7
EFSA Disclaimer 7
Information provided by the applicant 9
Food/constituent as stated by the applicant 9
Health relationship as claimed by the applicant 9
Wording of the health claim as proposed by the applicant 9
Specific conditions of use as proposed by the applicant 9
Assessment 10
1. Characterisation of the food/constituent 10
2. Relevance of the claimed effect to human health 10
3. Scientific substantiation of the claimed effect 11
Conclusions 15
Documentation provided to EFSA 15
References 15

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1924/2006[4] 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 07/09/2009.
- The scope of the application was proposed to fall under a health claim referring to disease risk reduction.
- The scientific evaluation procedure started on 15/09/2009.
- On 10-12/11/2009, 24–26/03/2010 and on 06/07/2010, EFSA requested the applicant to supplement additional particulars to accompany the application.
- The applicant submitted the responses to EFSA on 11/01/2010, 14/06/2010, 27/08/2010 and on 04/10/2010.
- During the meeting on 10-12/11/2010, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to *Lactobacillus casei* DN 114001 plus yoghurt cultures (Actimel®) and reducing the risk of *Clostridium difficile* associated diarrhoea by reducing the presence of *C. difficile* toxin.

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: *Lactobacillus casei* DN 114001 plus yoghurt cultures (Actimel®), and reduction of *C. difficile* toxins in the gut of patients receiving antibiotics and reduced risk of acute diarrhoea in patients receiving AB.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of *Lactobacillus casei* DN 114001 plus yoghurt symbiosis (Actimel®), a positive assessment of its safety, nor a decision on whether Actimel® 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.

[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.

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 France; 150 Boulevard Victor Hugo, 93589 Saint Ouen, Cedex, France.

The applicant indicates proprietary rights and confidentiality of unpublished study reports (Bulpitt, 2009; Bulpitt CJ and Hickson M, 2010; Houlder et al., 2009), an unpublished study protocol (D'Souza et al., 2003), an unpublished manuscript (Guimaraes et al., 2010), an abstract and the study synopsis of a study by Niborski et al. (2009) and an unpublished *in vitro* study (Danone Research, 2010). In addition the applicant claims proprietary rights of data related to the strain *Lactobacillus casei* DN-114 001, to the composition, manufacturing and quality control of Actimel®, stability information, and bioavailability data, presented in the dossier. The claim for proprietary rights include the published studies by Djouzi et al. (1997), Guerin-Danan et al. (1998), Guillemard et al. (2010), Oozeer et al. (2002, 2004, 2006), Parassol et al. (2005) and Rochet et al. (2006; 2008).

Food/constituent as stated by the applicant

The food is a fermented milk product containing *Lactobacillus casei* DN-114001 and yoghurt cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). This product is commercially available under the brand name Actimel®, the tested food.

Health relationship as claimed by the applicant

According to the applicant, the presence of *C. difficile* toxins in the gut is one of the risk factors of developing *Clostridium* associated acute diarrhoea. This risk factor occurs in combination with other factors such as ageing, antibiotic therapy, genetic background or impaired immune response.

According to the applicant the tested food decreases the presence of *C. difficile* toxins (the risk factor), in the intestinal tract and reduces the incidence of acute diarrhoea associated with their presence in the gut of susceptible ageing people.

Wording of the health claim as proposed by the applicant

Fermented milk containing the probiotic *Lactobacillus casei* DN-114001 and yogurt symbiosis decreases presence of *Clostridium difficile* toxins in the gut (of susceptible ageing people). Presence of *Clostridium difficile* toxins is associated with the incidence of acute diarrhoea.

Specific conditions of use as proposed by the applicant

The target population covered by this application encompasses a subgroup of the general adult population, i.e. ageing adults over 50 years old that are receiving antibiotic therapy.

The effective daily consumption of the tested food is 200 g of the fermented milk containing at least 10^8 cfu/g of *Lactobacillus casei* DN-114001 and yoghurt symbiosis (at least 10^7 cfu/g), within the frame of a balanced diet.

The product may not be suitable for individuals with a known cow's milk allergy.

Directions for preparation and/or use: The product should be consumed within the indicated shelf life and kept refrigerated during storage.

Assessment

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is Actimel®, a brand for a range of sweetened and flavoured fermented milks, as well as fat- and sugar-reduced flavoured versions. Actimel® is a fermented milk product with lactic acid bacteria (LAB) incorporated into a dairy matrix composed of cow's milk exclusively. The LABs in Actimel® are *Lactobacillus casei (Lc)* DN-114 001, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. Two main fermentation pathways are used to classify LAB genera as either homofermentative or heterofermentative. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are homofermentative - lactic acid is the primary end-product of fermentation, while *Lc* DN-114 001 is facultatively heterofermentative (end-products of fermentation, in addition to lactic acid, include acetic acid and carbon dioxide). According to the dossier provided, Actimel® contains at least 10^{10} cfu per 100 g (10^8 cfu/g) of *Lc* DN-114 001and 10^9 cfu (10^7cfu/g) of *L. delbrueckii subsp. bulgaricus* / *S. thermophilus* until the end of the shelf-life.

The strain *Lc* DN-114 001 has been characterised and was deposited at the Collection Nationale de Cultures de Microorganismes at the Institut Pasteur, Paris, France in 1994. *Lc* DN-114 001 is provided to a contract manufacturer and the master strain and working copies are kept in the applicant's strain collection. The full genome sequence of *Lc* DN-114 001 was obtained in 2005 and analysed in 2006. The full genome sequence is stored in ERGO™ (Bioinformatics suite comparative genomics analysis by Integrated Genomics, Inc) data base and is available upon request. The 16S-rDNA has been sequenced and the genotyping methods Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment-Length Polymorphism (AFLP), Multi Locus Sequence Typing (MLST) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) analyses (Diancourt et al., 2007) have been applied and confirm that the *Lc* DN-114 001 is a member of the *Lactobacillus paracasei subsp. paracasei* species which is in line with the classification of the Judicial Commission of the International Committee on Systematics of Bacteria (JCICSB, 2008).

The yogurt starter cultures present in the food have been identified either as *L. delbrueckii subsp. bulgaricus* DN-100 290 or *S. thermophilus salivarius subsp. thermophilus* DN-001 236 / DN-001 336 / DN- 001 460 by sequencing 16S rDNA and genotyping by AFLP, SDS page methods (internal reports are available upon request). The strains were deposited in the French National Collection of Cultures of Micro-organisms. The applicant provides sufficient information on the species and strain identification.

Since there is a wide range of flavoured fermented milks in the Actimel® brand, the composition of these products may be modulated with the addition of other ingredients, at a maximum incorporation level of 5 %, such as: fruit, fruit juice or plant concentrate, sugar (sucrose), fibres (oligofructose), thickener (tapioca starch), stabilizer (pectin), flavourings and acidity regulators (sodium citrate, citric acid).

The Panel considers that Actimel®, the food that is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "decreases the presence of *C. difficile* toxins (the risk factor), in the intestinal tract and reduces the incidence of acute diarrhoea associated with their presence in the gut of susceptible ageing people". The target population is adults over 50 years old receiving antibiotic treatment.

The Panel considers that treatment with certain antibiotics (AB) may be associated with *C. difficile* associated diarrhoea. The presence of *C. difficile* toxins in the gut may be associated with the incidence of acute diarrhoea.

The Panel considers that reducing the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant's literature search included studies between 1950 and July 2009 published in Medline, and between 1974 and July 2009 in EMbase using the online database aggregator Datastarweb. Details on the search strategy were given and included keywords such as Danone, Actimel®, DN-114001, *Lactobacillus casei*, probiotic, *Clostridium difficile*, human, patient, participant, clinical trial, human trial, clinical study, randomisation, double blind, control, cohort study, case control study, crossover study, multicenter trial, toxin, diarrhoea, acute diarrhoea, antibiotic associated diarrhoea (AAD).

In total the applicant indicated seven publications on human studies, three unpublished human studies, eight published and one unpublished non-human studies to be pertinent for the claimed effect. In addition the applicant provided 65 references related to *C. difficile*, AAD, the homeostasis of the intestine and possible mechanisms of "probiotics" which provided a general scientific background of this field. These 65 references did not refer to Actimel® or its bacterial strains. Three human studies referred to *C. difficile* associated diarrhoea (CDAD).

In an open non-controlled observational study with 213 elderly patients with a mean age of 88 (80 – 101) years in two geriatric care wards of a hospital, subjects received twice daily Actimel® for the duration of an antibiotic treatment course and 7 days thereafter (Houlder et al., 2009, unpublished). The study recorded the incidence of CDAD among patients in the geriatric wards for the period November 2007 to January 2008 and compared it with the historical incidence in the period of November 2006 to January 2007 when Actimel® had not been consumed. The Panel notes that this study was not controlled for factors other than the consumption of Actimel® that might have influenced the incidence of CDAD, e.g. differences in the use of antibiotics between the two periods. The Panel considers that no conclusion can be drawn from this study for the scientific substantiation of the claimed effect.

A RCT pilot study was designed to test the feasibility of a daily intervention with 2 x 100 ml per day of Actimel® and of a non-fermented acidified dairy drink to investigate the effect on the occurrence of AAD and of CDAD in elderly hospitalised patients receiving antibiotics (10 subjects per group). (Bulpitt, 2009, unpublished). The applicant commented in the study report that "no statistical comparison was performed in the context of a small (20 subjects) pilot study". The Panel agrees that this pilot study was underpowered to assess the effects of Actimel® on the incidence of AAD and CDAD. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claimed effect.

A RCT aimed to recruit 164 hospitalised patients of 50 or more years of age under antibiotic treatment to study the effect of daily 2 x 100 ml of the yoghurt drink Actimel® compared to 2 x 100 ml daily of a control product on the occurrence of AAD as the primary endpoint and CDAD as a secondary endpoint (Hickson et al., 2007; Bulpitt and Hickson, 2010 - unpublished). Other secondary study endpoints were lactobacillus counts in the stool, IgA levels specific to *C. difficile* in the stools, urinary indican ("as a marker for bacterial colonization in the gut"), use of AB (metronidazole and vancomycin to treat *C. difficile* infections), length of hospital stays, costs issues, C-reactive protein and white blood cell counts (WCC). The recruitment was stopped when 135 subjects were enrolled before reaching the target sample size of 164 subjects (82 per group) derived from the power calculation and predefined in the study protocol. The recruited patients had a mean age of 73.8 years.

Baseline characteristics were reported and were similar in both groups. One patient in each group was positive for *C. difficile* toxin at baseline but had no diarrhoea (neither patient subsequently developed diarrhoea).

The control product was Yazoo®, an ultra-high temperature treated, non-fermented, sweetened, flavoured dairy drink which was similar in colour and consistency to Actimel®. Test and control product were to be consumed twice a day during the course of AB and for one week after the course finished. As the products were packaged in bottles of different size (100 g Actimel® bottle versus 200 ml Yazoo® bottle), the hospital pharmacies removed the commercial labels and nursing staff were instructed to pour 100 ml of the respective products into a cup and to deliver to the patients in order to ensure blinding. According to the study protocol, patients who were discharged from the hospital before the end of the intervention were given adequate supplies of test and control products with instructions for use labelled on the bottles (D'Souza et al., 2003). The Panel considers that while the blinding procedure used in the hospital was adequate, the procedure used following discharge could have resulted in un-blinding of patients due to the different size of the bottles for the two products. Approximately 2/3 (at least 84 subjects) of the patients finished the intervention with the Actimel® and control product not in the hospital but at home.

"Diarrhoea" was defined as more than two liquid stools a day for three or more days in quantities in excess of normal for each patient. According to the applicant, diarrhoea during hospitalisation was reported by nurses and confirmed by one or more researchers and stool samples were taken. *Post*-discharge monitoring of diarrhoea was conducted via telephone by researcher (at least once per week) or with a questionnaire which was completed by the patient to record the presence or absence of diarrhoea. In the case of reported diarrhoea, the subjects were called to the hospital for a *C. difficile* toxin test of the stool. An ELISA kit, suitable to detect *C. difficile* toxin A and B was used. According to the study protocol, stool samples of all patients should have been tested at baseline, when diarrhoea was reported and at the end of the trial.

One hundred-thirteen patients completed the trial (drop-out rate: 16.3 %). The follow-up of 12 subjects of the Actimel® group and of 10 subjects of the control group could not be completed. In a complete- case analysis (per-protocol analysis), the authors reported AAD for 7 out of 57 patients in the Actimel® group and for 19 out of 56 patients of the control group. In none of the patients in the Actimel® group and in 9 out of 53 of the control group diarrhoea was associated with a stool sample positive for *C. difficile* toxins. This corresponds to a statistically significant 22 % (95 % CI: 7-37) and 17 % (95 % CI: 7-27) absolute risk reduction for the occurrence of AAD and CDAD, respectively. Data on *C. difficile* toxin testing from 4 subjects with reported diarrhoea were missing (1 in Actimel® group versus 3 in the control group). The applicant provided an analysis on the occurrence of AAD before and after hospital discharge. There was no significant difference between groups in the occurrence of AAD before hospital discharge (6 in the Actimel® group versus 7 in the placebo group), but a significant difference between groups was observed after the subjects were discharged from the hospital (0 in the Actimel® group versus 12 in the control group). According to the study report other secondary study endpoints (lactobacillus counts in the stool, *C. difficile* specific IgA levels in the stools, urinary indican, use of metronidazole and vancomycin to treat *C. difficile* infections, length of hospital stays, C-reactive protein and WCC) were not analysed due to insufficient data or techniques. The Panel notes that *C. difficile* toxins were only measured in patients who developed AAD and that there were no data on the effect of Actimel® on the occurrence of *C. difficile* toxins in the faeces of patients without AAD.

On a request of EFSA, the applicant provided a re-assessment of the data with and without data imputation for the non-completers, taking into account the covariates "number" and "type of prescribed AB" and the "duration of the AB treatment". For the data imputation, two scenarios were conducted assuming that a) none of the 22 drop outs had AAD and b) that 20 % in both groups had AAD. According to the applicant, 20 % represents the mean for the range of 5 – 35 % occurrence of AAD reported in the literature (referring to all ages, hospitalized and non-hospitalized patients). The

results remained statistically significant ($p < 0.05$) in favour of the test product for both scenarios. The Panel notes that statistical significance is sensitive to the treatment of the missing data and that no data imputation has been applied for more conservative scenarios than 20 % assumed occurrence of AAD.

The Panel notes a number of weaknesses in this study: potential bias through un-blinding of the patients to the products; the lack of data on the presence of *C. difficile* toxins (the risk factor) in the faeces of patients at the end of the intervention; no scenario has been applied for the missing data imputation for more conservative scenarios than 20 % assumed occurrence of AAD. The Panel considers that the weaknesses of this study limit its value as a source of data to substantiate the claim.

The applicant provided an *in vitro* study on the capability of *Lc* DN-114 001 to inhibit the growth of *C. difficile* (Danone Resarch, 2010, unpublished). According to the summary report the supernatant of *Lc* DN-114 001 was tested with the "overlay method" modified by Danone, for its capacity to inhibit the *in vitro* growth of *Salmonella thyphimurium, Listeria monocytogenes* and *C. difficile*. The strain *Lc* DN-114 001 was grown overnight and lactic acid neutralised culture supernatant was applied for 24 h to agar plates with pathogen colonies, in anaerobic conditions. Plates were then checked visually for the presence of growth inhibition areas around pathogens colonies. According to the data provided, the supernatant of *Lc* DN-114 001 tested positive for its capability to inhibit the growth of all three pathogens. The Panel considers that the results of this *in vitro* study do not predict the occurrence of such an inhibitory effect in humans.

Five human studies investigated the kinetics, metabolism and survival of *Lc* DN-114 001 and its impact on the human microbiota during its gastrointestinal transit (Guerin-Danan et al., 1998; Guimaraes et al., 2010, unpublished; Oozeer et al., 2006; Rochet et al., 2006, 2008). These studies demonstrated that *Lc* DN-114 001 can partially survive the gastrointestinal transit (Oozeer et al. 2006). Guimaraes et al. (2010, unpublished) demonstrated expression of 10 genes of *Lc* DN-114 001 in the faeces after a human gastrointestinal transit and did not find evidence for multiplication. Results from animal studies showed that *Lc* DN-114 001 can partially survive gastrointestinal transit in human flora-associated mice and rats and is excreted without detectable multiplication with the same kinetics as inert transit marker *Bacillus subtilis* spores (Djouzi et al., 1997; Oozeer et al., 2002; Oozeer et al., 2004). The Panel notes that human and animal data consistently showed partial survival of *Lc* DN-114 001 during its gastrointestinal passage without detectable multiplication.

Regarding possible mechanisms by which Actimel® could reduce the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins, the applicant suggested that Actimel® increased colonisation resistance against pathogens and inhibited pathogen proliferation by modulation of the intestinal microbiota, reduction of the gut epithelial permeability, modulation of gut epithelial receptors and induction of specific IgA production.

The applicant provided two human studies on the effect of Actimel® on gastrointestinal infections and gastrointestinal pathogens, but which did not refer to *C. difficile*. In a RCT with 1072 elderly volunteers consuming 2 x 100 ml Actimel® per day over 3 months, there was no difference in the incidence of common infectious diseases (primary endpoint), the incidence and duration of gastrointestinal infections, the occurrence of fever, the occurrence of pathogens, the number of prescribed medication and immunological parameters (among other secondary endpoints) (Guillemard et al., 2010). In another RCT, 250 young adult volunteers enrolled in firefighting training consumed 2 x 100 ml Actimel® per day over 7 weeks. There was no difference in the incidence of common infectious diseases (primary endpoint), the incidence of gastrointestinal infections, the occurrence of fever, the occurrence of pathogens in faeces and medication (among other secondary endpoints) (Danone, 2009; Niborski et al., 2009). The authors reported a significant increase of *L. paracasei* in faeces by a detection method which covered also the DN-114 001 strain. The Panel notes that no information has been provided on *C. difficile.* The Panel considers that these two RCTs do not support

an effect of Actimel® on reduction of gastrointestinal infections or gastrointestinal pathogens in humans.

Three other human studies addressed effects on the intestinal microbiota. In an uncontrolled human trial with 12 healthy subjects after 10 days of daily consumption of 300 ml Actimel® (Rochet et al., 2006) no statistically significant change occurred in either the dominant members of the faecal microbiota *(Atopobium, Bacteroides-Prevotella, Bifidobacterium, Clostridium coccoides, Faecalibacterium prausnitzii, enterobacteria* and *Lactobacillus–Enterococcus* groups), bacterial enzyme activities (α-Galactosidase, β–Galactosidase, β–Glucosidase, β–Glucuronidase, Neuraminidase, α-N-Acetylgalactosaminidase, β-N-Acetylgalactosaminidase, α-Fucosidase, Reductase, Azoreductase, β-N-Acetylglucosaminidase), pH and metabolites (such as short-chain fatty acids). This was confirmed by another human trial in 7 volunteers after 8 days of daily consumption of 300 ml Actimel® (Rochet et al., 2008) which did not find any statistically significant change of the proportions of 7 phylogenetic groups *(Bacterioides, Bifidobacterium genius, C. coccoides, Atopobium, Lactobacilli-Enterococci, Streptococci-Lactococci, and Enterobacteria*). Also Guerin-Danan et al. (1998) who studied the effect of a daily intake of 125 g Actimel® in 39 infants and young children of 10 -18 months of age, did not find any statistically significant modification of the number of total anaerobes, bifidobacteria, bacteroides, and enterobacteria, bacterial metabolites and the bacterial enzyme activities (β-Galactosidase, α-Glucosidase, Nitrate reductase, Nitroreductase, β-Glucuronidase, β-Glucosidase) in the faeces at the end of a 30-day intervention. Based on the parameters measured, the Panel considers that the three human studies do not provide evidence that Actimel® can modulate the human gut microbiota.

The applicant provided another two animal studies and three *in vitro* studies on possible effects of Actimel® or *Lc* DN-114 001 in model systems related to immune function and infection. Medici et al. (2005) investigated effects of Actimel® on the phagocytic activity of peritoneal macrophages, on IgA^+ cells in the intestine and on the colonisation of entero-invasive *E. coli* in a liver BALB/c mouse model. Guerin-Danan et al. (2001) studied effects of *Lc* DN-114 001 in SA11 rotavirus infected germfree suckling rats. The three *in vitro* experiments investigated the effect of spent culture supernatants of *Lc* DN-114 001 on the cell surface glycosylation and rotavirus infection of cultured intestinal epithelial cells (Freitas et al., 2003), the effect of *Lc* DN-114 001 on adhesion to and invasion of human intestinal epithelial cell lines by adherent-invasive *E. coli* isolated from Crohn's disease patients (Ingrassia et al., 2005) and the effects of *Lc* DN-114 001 on paracellular permeability of human colon epithelial T-84 cell cultures measured by electrical resistance and on the adhesion of enteropathogenic *E. coli* (EPEC) (Parassol et al., 2005). The Panel notes that none of these five studies investigated *C. difficile*. The Panel considers that the evidence provided does not establish that effects of Actimel® or *Lc* DN-114 001 observed in these animal and *in vitro* studies can predict the occurrence of effects on immune function or infection in humans.

In addition, the applicant provided 35 references on the homeostasis of the intestine and possible mechanisms of "probiotics" and 31 references on studies and reviews concerning the epidemiology, pathogenesis, risk factors, clinical symptoms, and therapy and management of nosocomial and community-acquired C. difficile infections, and diarrhoea associated with antibiotic therapy. A few articles concerned the impact of advanced age on the immune system and the human intestinal microbiota. These articles provide evidence that hospitalisation, antibiotic treatment and age above 65 years have been identified as the predominant risk factors for CDAD. Some references reported on other risk factors such as age over 60 years, exposure to subjects with CDAD, medication with proton pump inhibitors, anti-inflammatory, immunosuppressive and anti-neoplastic drugs (Barbut and Petit, 2001; Karlström et al., 1998; Kuijper et al., 2008; McFarland, 2008; Wiström et al., 2001). The Panel notes the risk factors including the age range of individuals with CDAD described in the literature.

In weighing the evidence, the Panel took into account that human and animal studies showed partial survival of *Lc* DN-114 001 during its gastrointestinal passage, that one human intervention study with Actimel® which showed a statistically significant risk reduction for CDAD had considerable

limitations, that there were only limited data on the effect of Actimel® on the reduction *C. difficile* toxins (risk factor) in humans, that one study which showed an inhibitory effect of *Lc* DN-114 001 on the growth of *C. difficile in vitro* does not predict the occurrence of an effect against *C. difficile* in humans, that five further human studies do not support the proposed mechanisms by which Actimel® could exert the claimed effect, and that the evidence provided from a further two animal and three *in vitro* studies does not establish that effects of Actimel® or *Lc* DN-114 001 in these model systems related to immune function and infection can predict the occurrence of such effects in humans.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of Actimel® and reduction of the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins.

CONCLUSIONS

- The food constituent, Actimel®, that is the subject of the claim is sufficiently characterised.
- The claimed effect is to decrease the presence of *Clostridium difficile* toxins in the gut of susceptible elderly people. The target population encompasses a subgroup of general adult population, i.e. ageing adults over 50 years old that are receiving antibiotics.
- The Panel considers that the reduction of *C. difficile* toxin in the gut of patients receiving antibiotics maybe beneficial. *C. difficile* toxin in the gut of patients receiving antibiotics is a risk factor in the development of AAD.
- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of Actimel® and reduction of the risk of *C. difficile* diarrhoea by reducing the presence of *C. difficile* toxins.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on fermented milk containing *Lactobacillus casei* DN-114 001 plus yoghurt symbiosis (Actimel®) and "decreases presence of *Clostridium difficile* toxins in the gut of susceptible ageing people" pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0260_FR). September 2009. Submitted by Danone France.

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