European Food Safety Authority
Unit on Dietetic products, Nutrition and Allergies

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Mr. Basil Mathioudakis
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Head of Unit
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Subject: Response to comments with regard to the EFSA opinion on the scientific substantiation of a health claim related to **"Immunofortis®" and "strengthening of the baby's immune system"** (EFSA-Q-2008-106) pursuant to Article 14 of Regulation (EC) No 1924/2006.

Dear Mr. Mathioudakis,

Thank you for informing EFSA about the comments the European Commission received from the applicant Danone Baby Nutrition and other experts related to the EFSA NDA opinion on "Immunofortis® " and "strengthening of the baby's immune system" (EFSA-Q-2008-106) , which was adopted by the NDA panel on 4 December 2009.

EFSA has reviewed the comments and shared them with the two vice chairs of the EFSA NDA panel, Prof. Henk van Loveren and Prof. Hildegard Przyrembel, and we would like to clarify the specific substantive issues raised by the applicant and the author of the key human intervention study. Comments received from four other sources (Professors Calder, Walker, Decsi and Dupont) are generally similar to those submitted by the applicant and are covered in this response.

1. Methodology of the human intervention studies, in particular the study related to the incidence of atopic dermatitis and infections

Outcome of the clinical intervention study

With regards to questions raised by the Panel on methods for diagnosis of atopic dermatitis (AD) (the "allergic" nature of clinically diagnosed dermatitis, wheezing and urticaria were not assessed) the applicant states in the comments that this has been performed by highly skilled paediatricians using internationally accepted standards and that serological confirmation is not feasible in clinical studies in infants. Accepting the limitations of clinical studies in infants, it is noted that the claim related to initiation of appropriate immune responses for which the Panel notes that clinically observed manifestations of atopic dermatitis, recurrent wheezing and allergic urticaria might indicate a change in immune response. However, the symptoms reported do not necessarily indicate an allergic mechanism. The observation of the Panel was that data on commonly accepted

immunological parameters might have been supportive of the role of the immune system for the symptoms on which clinical diagnosis of allergic manifestations was made.

With regards to questions raised by the Panel on methods for diagnosis of infection the applicant states in the comments that this has been performed by highly skilled paediatricians and confirmed by a fever of at least 38.5C. Accepting the limitations of clinical studies in infants, it is noted that the claim related to initiation of appropriate immune responses for which the Panel notes that clinically diagnosed infection or fever of at least 38.5C observed by parents might indicate a change in immune response. The observation of the Panel was that data on microbiological/serological parameters in biological samples might have been supportive of the role of the immune system for the symptoms on which diagnosis of infection was made.

With regards to questions raised by the Panel on criteria used by paediatricians for antibiotic prescription, and whether those criteria where applied uniformly in the intervention and control groups, the applicant states in the comments that the randomized, double blind nature of the study would ensure application of the criteria uniformly and without bias. EFSA accepts this but considers that more information on the criteria used for antibiotic prescription might have been supportive of the use of this parameter as an indicator of infectious episodes that might indicate a change in immune response.

Statistical analysis

With regards to the lack of a correction for multiple testing, the applicant states in the comments that the primary clinical outcome parameter was cumulative incidence of "atopic dermatitis" during the first 6 months of life (as described Moro et al. 2006) and the secondary parameter was "incidence of infectious episodes" (as described in Arslanoglu et al. 2007). The applicant indicates that the confusion between primary and secondary outcomes was clarified in a letter to the journal editor (Arslanoglu et al., 2008b).

With regard to "incidence of infectious episodes", it is clear from Arslanoglu et al. (2007, 2008) and from the application submitted to EFSA that there were multiple outcome measures and that no correction for multiple testing was used in the statistical analysis.

With regard to "incidence of AD", while Moro et al (2006) report on the outcome at 6 months for "cumulative incidence of atopic dermatitis", the report on the outcome at 2 years (Arslanoglu et al. 2008) states that primary outcomes were "cumulative incidence of allergic manifestations: atopic dermatitis, recurrent wheezing, and allergic urticaria" which were recorded at 10 time points over the 2 years, including monthly over the first 6 months. However, data on recurrent wheezing and allergic urticaria were not presented for the first 6 months, and data were not presented on incidence of any of the allergic manifestations for any of the 8 intermediate time points over the 2 years.

Taking into account the comments from the applicant and the data presented, EFSA confirms that it considers that the lack of a correction for multiple testing contributes to the weakness of the study.

2. Relevance of gut microbiota composition and metabolic activity as part of the first line of defence resulting in defence against pathogens

Increase in number/proportion of bifidobacteria or lactobacilli

The panel acknowledged in its opinion that a number of studies showed a significant increase in the number/proportion of bifidobacteria or lactobacilli in stool samples of infants after consumption of scGOS/lcFOS. However the Panel considered that the evidence provided does not establish that an increased number/proportion of bifidobacteria or lactobaccili in faeces represents *per se* a beneficial physiological effect.

Inhibition of pathogenic bacteria

With regards to inhibition of pathogenic bacteria the applicant states in the comments that several peer reviewed studies submitted in the application reported a significant reduction of potentially pathogenic bacteria, most notably a significant decrease in the proportion of the *Clostridium histolyticum/C. lituseburense* group (that includes the pathogens *Clostridium difficile and Clostridium perfringens)* (Scholtens et al. 2008a,b; Costalos et al. 2008) and *Enterobacteriacea* (Scholtens et al. 2008b; Magne et al., 2008). The applicant also suggests that the sum of the relevant pathogenic bacteria should be taken into account as a relevant end-point.

Five references were provided to support this aspect, which were carefully reviewed by the Panel (Costalos et al., 2008; Knol et al, 2005a; Magne et al., 2008; Scholtens et al., 2008a; 2008b). For four of the five studies (Costalos et al. 2008; Scholtens et al. 2008a,b; Magne et al., 2008) data were provided on groups of bacteria only but not on the individual potentially pathogenic species. Accepting that changes in potential pathogenic microorganisms in faeces might indicate a change in immune response, the NDA panel noted that the evidence provided does not establish that a decrease in a group of bacteria, like *Clostridium spp* (which may comprise both non-pathogenic and potentially pathogenic bacteria) is an appropriate measure of potentially pathogenic bacteria. Additional limitations embedded in the studies of Costalos et al. (2008) and Scholtens et al. (2008a) are referred to in the opinion.

In one study (Knol et al. 2005a) individual potentially pathogenic species and groups of bacteria that included pathogens (as well as non-pathogens) were investigated. However, data were presented on the sum of bacteria of these different groups and species only and no data were provided for the individual species of potentially pathogenic bacteria. The Panel considered that the evidence provided does not establish that the sum of bacteria of these different groups and species is an appropriate measure of potentially pathogenic bacteria.

3. The evidence supporting a biologically plausible mechanism by which Immunofortis® (scGOS/lcFOS (9:1)) exerts the claimed effect

The applicant has proposed several possible mechanisms by which scGOS/lcFOS could contribute to the initiation of an appropriate immune response. It is suggested that a biologically plausible mechanism for defence against pathogens is supported by changes in secretory immunoglobulin A, the number/proportion of bifidobacteria or lactobacilli, pH and SCFA in stool samples of infants. In addition, the development of an anti-allergic immunoglobulin profile and a possible role for immunoglobulin free light-chain (Ig-fLC) has been suggested as biologically plausible mechanisms for initiation of an appropriate immune response related to protection against allergic disorders.

All of these possible mechanisms were considered by the Panel which concluded that the evidence presented in support of a biologically plausible mechanism by which scGOS/lcFOS could exert the claimed effect is not convincing. The evidence provided does not establish an effect of scGOS/lcFOS on faecal secretory immunoglobulin A. In addition the evidence provided does not establish that changes observed in the number/proportion of bifidobacteria or lactobacilli, pH or SCFA in stool samples of infants were associated with initiation of an appropriate immune response for defence against pathogens. (Although considered by the Panel, the evidence on a possible role for pH and SCFA was not referred to in the opinion). Similarly, the evidence provided does not establish that changes observed in the immunoglobulin profile or the plasma concentration of immunoglobulin free light-chain (Ig-fLC) of infants were associated with initiation of an immune response that protected against allergic manifestations.

4. The relevance of the animal studies supporting the immunological effects of scGOS/lcFOS (9:1)

The applicant has suggested that the data provided on immunological effects of scGOS/lcFOS in animal studies provide supportive evidence for initiation of appropriate immune response and have been validated by human studies (e.g. Van Hoffen, 2009). However, as indicated above, the evidence provided in human studies does not establish that changes in immunoglobulins in infants were associated with allergic manifestations. In the absence of such human data the Panel concluded that the evidence provided in the animal studies submitted does not predict the occurrence of an effect of scGOS/lcFOS on the initiation of appropriate immune responses in humans.

The applicant has also suggested that immunomodulatory effects of scGOS/lcFOS observed *in vitro* provide supportive evidence for initiation of appropriate immune response. However, the evidence provided does not establish that effects in such model systems are predictive of beneficial immunomodulatory effects in humans.

5. Assessment methodology of EFSA

The applicant has questioned the methodology adopted by EFSA for assessment of the application. EFSA wishes to point out that the assessment of this application followed the criteria and procedures specified in the Regulation that have been explained in EFSA's guidance to applicants. In this regard, the opinion has indicated the reasons that some of the presented studies were not considered pertinent for substantiation of the claim and how the different types of scientific data were assessed and taken into account in the weighing of the evidence.

6. Conclusions of review of comments received

In conclusion, having taken into account the specific substantive issues raised by the applicant and the author of the key human intervention study (covering also the comments received from four other sources) we wish to reiterate the overall conclusion of the Panel, i.e. that the information provided is insufficient to establish a cause and effect relationship between the consumption of Immunofortis® and the claimed effect of initiation of appropriate immune responses including the defence against pathogens.

I hope you will find our comments useful.

Yours sincerely,

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Cc: Noel Griffin, Christophe Didion, Christina Antoniou, Francesco Felice Carlucci, Sabine Osaer