DRAFT SCIENTIFIC OPINION

Guidance on the scientific requirements for health claims related to the gastro-intestinal tract, the immune system, and defence against pathogenic microorganisms [1]

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

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ABSTRACT

The European Food Safety Authority (EFSA) has asked the Panel on Dietetic Products, Nutrition and Allergies (NDA) to revise the guidance on the scientific requirements for health claims related to gut and immune function, which was published in 2011. The revision takes into account the outcome of a public consultation on a discussion paper together with new scientific evidence available to the NDA Panel and the experience gained to date with the evaluation of health claim applications in the areas of the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms. The guidance presents examples drawn from evaluations to illustrate the approach of the NDA Panel in the evaluation of health relationships and outcome variables which may be acceptable in these areas, as well as the conditions under which they may be acceptable. It is not intended to include in the document an exhaustive list of beneficial effects and studies/outcome variables which could be acceptable. The reason is that defining the conditions under which health relationships and outcome variables for claimed effects may be acceptable is generally possible only in the context of specific applications, which are often unique and technically complex. A better understanding of the approach of the NDA Panel could help applicants in preparing applications on health relationships and related outcome variables. This draft guidance was discussed and endorsed by the NDA Panel on 10 December 2014 for release for public consultation before finalisation.

KEY WORDS

health claims, scientific requirements, gut and immune, microorganisms, consultation

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SUMMARY

The European Food Safety Authority (EFSA) has asked the Panel on Dietetic Products, Nutrition and Allergies (NDA) to revise the guidance on the scientific requirements for health claims related to gut and immune function, which was published in 2011.

The revision takes into account the outcome of a public consultation on a discussion paper together with new scientific evidence available to the NDA Panel and the experience gained to date with the evaluation of health claim applications in the areas of the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms. The guidance document has been structured taking into consideration the comments and the request for clarification received during the public consultation on the discussion paper.

This guidance is intended to assist applicants in preparing their applications for the authorisation of health claims related to the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms. The document presents examples drawn from past and on-going evaluations to illustrate the approach of the NDA Panel in the evaluation of health relationships and outcome variables which may be acceptable in these areas, as well as the conditions under which they may be acceptable. It is not intended to include in the document an exhaustive list of beneficial effects and studies/outcome variables which could be acceptable. The reason is that defining the conditions under which health relationships and outcome variables for claimed effects may be acceptable is generally possible only in the context of specific applications, which are often unique and technically complex. A better understanding of the NDA Panel approach could help applicants in preparing applications on health relationships and related outcome variables.

The draft guidance document was discussed and endorsed at the NDA Plenary meeting of December 2014, and is released for public consultation before finalisation.

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BACKGROUND AS PROVIDED BY EFSA

Regulation (EC) No 1924/2006[4] harmonises the provisions related to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. According to the Regulation, health claims should be only authorised for use in the Community after a scientific assessment of the highest possible standard to be carried out by EFSA.

Owing to the scientific and technical complexity of health claims, the EFSA Panel on Dietetic products, Nutrition and Allergies (NDA Panel) has placed considerable focus on developing scientific criteria for substantiation of health claims and has published guidance on scientific substantiation of health claims since 2007[5].

To date, over 570 scientific opinions related to health claims have been published and the Panel notes that additional health relationships and outcome measures for specific claimed effects have been considered in the context of specific applications.

Based on experiences gained with the evaluation of health claims, and to further assist applicants in preparing and submitting their applications for the scientific evaluation of health claims, the NDA Panel considers it necessary to update existing guidance documents, and/or to develop new guidance documents, on the scientific requirements for the substantiation of health claims, if considered appropriate.

The NDA Panel also emphasises the importance of engaging in consultation with experts/stakeholders in the process of updating existing guidance documents and/or developing new guidance documents.

It is proposed to undertake this task in a stepwise manner, taking into account the experience gained and new scientific evidence available to the NDA Panel, including outcomes of public consultations with experts/stakeholders.

Owing to a high demand from stakeholders and questions received from applicants requesting clarification related to gut and immune function claims, it is proposed to start with updating the existing Guidance document on the scientific requirements for health claims related to gut and immune function[6].

TERMS OF REFERENCE AS PROVIDED BY EFSA

The NDA Panel is requested by EFSA to update the existing Guidance document on scientific requirements for health claims related to gut and immune function.

In this context, as an initial step, the Panel is requested to issue a statement to be released for public consultation to gather views from experts/stakeholders in the field before proceeding with the updating of the guidance document. The statement shall point out the issues to be covered in the guidance document, propose recommendations for the updating of the guidance document, and propose a timetable for the release of draft and final guidance.

As a second step, taking into account the experience gained and new scientific evidence available to the NDA Panel, including the outcome of the public consultation on the statement, the Panel is requested to update and draft the Guidance document to be released for public consultation before finalisation.

[4] 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.
[5] http://www.efsa.europa.eu/en/nda/ndaclaims.htm
[6] http://www.efsa.europa.eu/en/efsajournal/pub/1984.htm

Before the adoption of the guidance document by the NDA Panel, the draft guidance needs to be revised taking into account the comments received during the public consultation.

A technical report on the outcome of the public consultation on the guidance document shall be published, in which comments received on the statement shall be included.

ASSESSMENT

1. Introduction

The Guidance on the scientific requirements for health claims related to gut and immune function (EFSA-Q-2010-01139)[7] laid down recommendations on specific issues that need to be addressed in the applications submitted for the substantiation of health claims related to the gastro-intestinal tract and the immune system. The guidance, published in April 2011, was based on the experience gained by the EFSA Panel on Dietetic products, Nutrition and Allergies (NDA Panel) with the earlier evaluation of health claims in these areas. Since then, the NDA Panel has evaluated additional health claim applications related to gut and immune function, and notes that new health relationships and outcome measures have been considered in the context of specific applications. The NDA Panel also notes that a considerable number of requests for clarification have been received from applicants related to gut and immune function claims, and therefore considers it necessary to update the Guidance document on scientific requirements for health claims related to gut and immune function[8].

The NDA Panel also emphasises the importance of engaging in consultation with experts from academia and with stakeholders in the process of updating existing guidance documents and/or developing new guidance documents. It is proposed to undertake this task in a stepwise manner, taking into account new scientific evidence available to the NDA Panel and based on the experience gained with the evaluation of health claims, and on the outcome of public consultations.

Thus, the present draft guidance takes into account the outcome of a public consultation on a discussion paper together with new scientific evidence available to the NDA Panel and the experience gained to date with the evaluation of health claim applications in the areas of the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms. The draft guidance document has been structured taking into consideration the comments and the request for clarification received during the public consultation on the discussion paper. A report on the outcome of the public consultation on the discussion paper, together with the comments received, has been published on the EFSA website[9].

It is anticipated that the revision will benefit both industry (by providing clearer requirements) and evaluators of health claims (through receiving better applications).

2. Objectives and scope

This guidance is intended to assist applicants in preparing their applications for the authorisation of health claims related to the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms.

The guidance presents examples drawn from past and on-going evaluations to illustrate the approach of the NDA Panel in the evaluation of health relationships and outcome variables which may be acceptable in these areas, as well as the conditions under which they may be acceptable. A better understanding of such an approach could help applicants in preparing applications on health relationships and related outcome variables. The guidance does not intend, however, to provide an exhaustive list of beneficial physiological effects and studies/outcome variables which could be acceptable, or address health relationships and related outcome measures which have not yet been considered by the Panel in the context of a particular application. The reason is that defining the conditions under which health relationships and outcome variables for claimed effects may be acceptable is generally possible only in the context of specific applications, which are often unique and technically complex (e.g. health relationships and outcome variables which may be acceptable in

[7] http://www.efsa.europa.eu/en/efsajournal/pub/1984.htm
[8] http://www.efsa.europa.eu/en/efsajournal/pub/1984.htm
[9] http://www.efsa.europa.eu/en/supporting/pub/758e.htm

the context of a particular application may not be so in the context of another application with, for example, a different target population).

It is also not within the scope of this guidance to provide detailed instructions on the design of scientific studies, but rather to give general indications to applicants of the types of studies, study groups and outcomes that may be appropriate for the substantiation of health claims. The NDA Panel considers what is generally accepted in the research field (e.g. guidelines published by scientific societies based on rigorous methodological approaches) and consults experts in the discipline, as appropriate. It is the responsibility of the applicant to ensure that the studies are performed according to standards that are generally accepted by experts in the relevant field.

It is intended that the guidance will be kept under review and will be amended and updated as appropriate in the light of experiences gained from evaluation of additional health claim applications.

Issues which are related to substantiation that are common to health claims in general (e.g. wording of claims, handling of confidential and proprietary data) are addressed in the general guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims[10].

This document should be read in conjunction with Regulation (EC) N° 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on foods[11], the Guidance on the implementation of Regulation (EC) No 1924/2006 of the Standing Committee on the Food Chain and Animal Health for comparative nutrition claims made on foods[12], and all other pertinent elements outlined in the general guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims[13] and currently available[14] and future guidelines and regulations, as applicable.

3. General principles

3.1. Characterisation of the food/constituent

Health claims related to the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms have been proposed for food/constituent(s) (including microorganisms). The NDA Panel considers whether the specific food/constituent is sufficiently defined and characterised, to establish that the studies provided for substantiation of the claim were performed with the food/constituent for which the claim is proposed. There should be sufficient definition of the food/constituent used in the studies provided for substantiation of the claim. Characterisation should also be sufficient to allow the definition of appropriate conditions of use[15]. It is the responsibility of the applicant to provide this information along with information regarding manufacturing processes, where applicable, in order to show consistency in the final product for those characteristics considered to be pertinent to the claimed effect.

The NDA Panel considers whether the information provided includes those characteristics considered pertinent to the claimed effect, i.e. those characteristics which may influence the specific physiological effect that is the basis of the claim.

[10] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011. General guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims. EFSA Journal, 9(4):2135, 24 pp.
[11] 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.
[12] Guidance on the implementation of Regulation (EC) No 1924/2006 on nutrition and health claims made on foods – Conclusions of the Standing Committee on the Food Chain and Animal Health, 14 December 2007.
[13] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011. General guidance for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims. EFSA Journal, 9(4):2135, 24 pp.
[14] http://www.efsa.europa.eu/en/nda/ndaguidelines.htm
[15] Although not required for substantiation of a claim, characterisation should also be sufficient to allow control authorities to verify that the food/constituent which bears a claim is the same one that was the subject of a Community authorisation.

- If the claim is for an individual constituent, the source and specification (e.g. physical and chemical properties) should be provided. The substantiation of the claim is based on studies performed with this constituent.

- If the claim is for a specific formulation or a fixed combination of constituents, then studies are needed on this specific formulation or combination. If individual constituent(s) in the specific formulation have an established role on the claimed effect, the NDA Panel also considers whether: i) the effect could be explained by the individual constituent(s), regardless of the source; ii) other constituent(s) in the specific formulation are required for/contribute to the claimed effect.

- For a food category (e.g. "dairy"), the NDA Panel considers whether the information provided sufficiently addresses the variability between individual foods for those characteristics considered pertinent to the claimed effect.

- For plant products, the NDA Panel considers whether the information provided includes the scientific name (e.g. Punica granatum *L.*), the part used (e.g. root, leaf, seed), complete specifications of the manufacturing process (e.g. dried, hydroalcoholic extraction), and how the product is standardised (e.g. by its content of one or more specific constituents).

- For microorganisms (e.g. bacteria and yeast), see Section 3.1.1 below.

3.1.1. Characterisation of microorganisms at strain level

Health claims have been made on microorganisms (e.g. bacteria and yeast). Correct identification of the bacterium's and yeast's species and strain is of critical importance, as the observed effects in the host are species and strain specific, unless the contrary is demonstrated.

Species identification and sufficient characterisation (genetic typing) at strain level, by using internationally accepted molecular methods is needed. In addition, strains should be named according to the International Code of Nomenclature. It is strongly recommended that strains are deposited in an internationally recognised culture collection (with access number) for control purposes.

Characterisation of bacteria[16, 17] - The Panel uses the following criteria for characterisation of bacteria, which are the subject of health claims:

- Species identification by DNA-DNA hybridisation or sequence analysis of robust taxonomic markers (e.g. 16S rRNA gene sequencing).

- Strain identification by DNA macrorestriction followed by pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA analysis (RAPD), or other internationally accepted genetic typing molecular methods e.g. Amplified fragment length polymorphism (AFLP), optical mapping, etc.

Only when these two criteria are fulfilled is the bacterium considered to be sufficiently characterised.

Characterisation of yeasts[18] - The Panel uses the following criteria for the characterisation of yeasts which are the subject of health claims:

- Species identification by restriction fragment length polymorphism analysis (RFLP) (e.g. RFLP of PCR products of the 5.8S rDNA internal transcribe spacer [ITS] region) or by sequencing analysis of DNA taxonomic markers (e.g. the D1 and D2 domains of 26S rDNA or ITS regions).

[16] http://www.efsa.europa.eu/en/efsajournal/pub/1247.htm
[17] http://www.efsa.europa.eu/en/efsajournal/pub/1470.htm
[18] http://www.efsa.europa.eu/en/efsajournal/pub/1470.htm

- Strain identification by chromosome length polymorphism analysis by PFGE, RAPDs, microsatellite DNA polymorphism analysis or other internationally accepted genetic typing molecular techniques.

Only when these two criteria are fulfilled is the yeast considered to be sufficiently characterised.

In the case of combination of several bacteria and/or yeasts, the Panel considers that if one microorganism used in the combination is not sufficiently characterised, the combination proposed is not sufficiently characterised.

The NDA Panel recommends that applicants provide sufficient information complying with the above-mentioned criteria for the characterisation of microorganisms.

3.1.2. Characterisation of microorganisms and other food constituents in relation to the claimed effect

Food/constituents cannot be characterised on the basis of the claimed effect (e.g. non-cariogenic carbohydrates, antioxidant foods, microorganisms which contribute to the defence against pathogens in the respiratory tract). In specific circumstances, however, the food/constituent(s) could be characterised on the basis of a property which could explain their contribution to the claimed effect (i.e. when the mechanism by which the claimed effect is achieved is known). For example, yoghurt starter cultures contribute to improved lactose digestion[19] by producing β-galactosidase. In this case, characterisation of the starter cultures of yoghurt at species level is considered sufficient in relation to the claimed effect because all the strains within the species share the property of producing β-galactosidase, which is the mechanism by which they contribute to improved lactose digestion.

3.2. Characterisation of the target population for a claim and of the claimed effect

3.2.1. Characterisation of the target population for a claim

The target population is the population group for which health claims are intended. The NDA Panel considers that the target population for the claim is the *general (healthy) population or specific subgroups thereof*, e.g. men, women, elderly subjects, physically active subjects and pregnant women are part of the general population and as such can be the target population for a claim and the study population.

With respect to *children*, the Commission guidance on the implementation of Regulation (EC) 1924/2006[20] clarifies the term "children" and the conditions and requirements for health claims targeting children.

As per Article 7(3) of Regulation (EU) No 1169/2011[21], the food information to consumers shall not attribute to any foodstuff the property of preventing, treating or curing a human disease; therefore health claims made on foods cannot refer to the treatment of a disease, and thus *subjects with a disease* cannot be the target population for a claim.

Subjects under medical treatment for a disease could be the target population for a claim, even if the medical (e.g. pharmacological) treatment affects the target function for the claim. However, as outlined in the Commission's summary report of the Standing Committee meeting dated 13 June 2014[22], the acceptability of applications for authorisation of claims which target groups under medical treatment and which relate to side effects of the treatment are to be assessed on a case by case basis by the Member States. In this respect, applicants are invited to check the admissibility of the target

[19] http://www.efsa.europa.eu/en/search/doc/1763.pdf
[20] http://ec.europa.eu/food/food/labellingnutrition/claims/guidance_claim_14-12-07.pdf
[21] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&from=EN
[22] http://ec.europa.eu/food/committees/regulatory/scfcah/general_food/docs/sum_20140613_en.pdf

population for the claim with the recipient Member State at the earliest possible stage of their consideration regarding the submission of an application for authorisation of a health claim.

3.2.2. Characterisation of the claimed effect

According to Regulation (EC) No 1924/2006, the use of health claims shall only be permitted if the food/constituent, for which the claim is made, has been shown to have a beneficial physiological effect (i.e. a benefit for a specific function of the body).

In assessing each claim, the NDA Panel makes a scientific judgement on whether the claimed effect is considered to be a beneficial physiological effect in the context of the specific claim, as described in the information provided by the applicant and taking into account the population group for which the claim is intended.

3.2.2.1 Characterisation of the claimed effect for function claims

For function claims, a beneficial physiological effect may relate to the maintenance, reduced loss or improvement of a function. To allow a scientific evaluation by the NDA Panel, the claimed effect needs to fulfil the following requirements:

3.2.2.1.1 The claimed effect is defined

In assessing each specific food/health relationship, which forms the basis of a health claim, the Panel considers whether the claimed effect refers to a specific function of the body (i.e. it is not general and non-specific) as required by Regulation (EC) No 1924/2006. Examples of claims which were not considered by the NDA Panel as sufficiently defined for a scientific evaluation include *"gut health", "natural defences", "strengthen the immune system", "maintenance of a normal immune system", "normal development of gut function", "normal digestion".*

3.2.2.1.2 The claimed effect is beneficial for the target population

In assessing each specific food/health relationship, the Panel also considers whether the claimed effect is a beneficial physiological effect for the target population (the general population or population subgroups thereof) for which the claim is intended. For example, "a reduction of gastric acid levels"[23] or "a reduction of inflammation"[24] could represent therapeutic targets for the treatment of some disease conditions, but are not considered beneficial physiological effects for the general population.

3.2.2.1.3 The claimed effect refers to a specific function of the body and can be measured *in vivo* in humans

In order to allow a scientific evaluation by the NDA Panel, the claimed effect needs to refer to a function of the body and be specific enough to be testable and measurable *in vivo*[25] in humans by generally accepted methods, except for health claims on essential nutrients (as explained in Section 3.4 of this guidance document). In this context, it should be noted that:

a) claimed effects, which are considered as beneficial physiological effects, may not allow a scientific evaluation by the NDA Panel in the context of a particular application if no generally accepted methods for the measurement of the outcome variable(s) of interest *in vivo* in humans have been provided. An example is the lack of generally accepted methods for the measurement of the inhibition of adhesion of P-fimbriated *E. coli* to uroepithelial cells *in vivo* in humans, even though this particular effect was considered a beneficial physiological effect in the context of a particular application for a claim on reduction of bacterial colonisation of the urinary tract by inhibition of the adhesion of P-

[23] http://www.efsa.europa.eu/en/efsajournal/doc/1472.pdf
[24] http://www.efsa.europa.eu/en/efsajournal/doc/2059.pdf
[25] It includes the measurement of functional outcome variables *in vivo* and the measurement (*ex vivo)* of outcome variables in biological samples following an intervention *in vivo.*

fimbriated *E.coli* to uroepithelial cells. The reasons for the Panel's conclusions can be found in the published opinion[26].

b) changes in outcome variable(s), which can be measured *in vivo* in humans by generally accepted methods may not be considered beneficial physiological effects *per se* if they do not refer to a benefit on a specific function of the body, and thus cannot be the claimed effect (i.e. constitute the only basis for the scientific substantiation of a health claim).

Some examples of outcome variable(s) which can be measured *in vivo* in humans by generally accepted methods but do not refer to a benefit on specific functions of the body and thus cannot constitute the only basis for the scientific substantiation of a health claim include:

i) changes in stool pH and short-chain fatty acid production (including butyrate) in the gut;

ii) changes in the composition of the gut microbiota;

iii) changes in the structure of the intestinal epithelium;

iv) changes in markers of inflammation (including markers of chronic, subclinical inflammation), such as interleukins or C-reactive protein;

v) changes in immune markers, e.g. numbers of various lymphoid subpopulations in the circulation, proliferative responses of lymphocytes, phagocytic activity of phagocytes, lytic activity of natural killer cells and cytolytic T cells, production of cellular mediators, serum and secretory immunoglobulin levels, delayed-type hypersensitivity responses, etc.

Changes in some of these outcome variables could, however, be proposed as part of the mechanisms by which a food may exert the claimed effect, i.e. induce a beneficial change on a specific function of the body (e.g. maintenance of normal defecation, improved absorption of essential nutrients, or defence against pathogens).

However, in specific circumstances, changes in outcome variable(s) measured *in vivo* in humans, and which do not refer to a specific function of the body directly, may be the claimed effect if evidence is provided that changes in such variable(s) generally induce a beneficial change in a specific function of the body. An example is the reduction of excessive intestinal gas accumulation, which does not refer directly to a benefit on a specific function of the body, but for which evidence has been provided that the change of the variable generally induces a beneficial change in a specific function of the body, i.e. reducing gastrointestinal discomfort (see Section 4.1.3).

3.2.2.2 Characterisation of the claimed effect for disease risk reduction claims

For reduction of disease risk claims, the beneficial physiological effect (which Regulation (EC) No 1924/2006 requires to be shown for the claim to be permitted) is the reduction (or beneficial alteration) of a risk factor for the development of a human disease (not reduction of the risk of disease).

Whether or not the alteration of a factor is considered to be beneficial in the context of a reduction of disease risk claim depends on the extent to which it is established that:

- The factor is an independent predictor of disease risk (such a predictor may be established from intervention and/or observational studies);
- The relationship of the factor to the development of the disease is biologically plausible.

[26] http://www.efsa.europa.eu/en/efsajournal/doc/3082.pdf

If there is strong evidence that there is (i) an independent association between the risk factor and the incidence of the disease, including (ii) a strong evidence for the biological basis through which the risk factor can contribute to the development of the disease, and (iii) evidence that a given modification of the risk factor generally reduces the risk of disease, a given modification of the risk factor may be considered beneficial in the context of a reduction of disease risk claim. In this case, evidence that the dietary intervention induces a given modification on the risk factor for the disease would be sufficient for the scientific substantiation of the claim.

If the evidence is not as strong (e.g. there is evidence for an independent association between the risk factor and the incidence of the disease and for the biological basis through which the risk factor can contribute to the development of the disease, but no evidence that a given modification of the risk factor generally reduces the risk of disease), a given modification of the risk factor may still be considered a beneficial physiological effect in the context of a reduction of disease risk claim. In this case, evidence needs to be provided that a given modification of the risk factor is accompanied by reduced incidence of the disease following a specific dietary intervention, preferably in the same studies (e.g. by consuming the food/constituent for which the claim is made) (see also section 5).

3.3. Human studies submitted for the scientific substantiation of health claims

As human data are central for the substantiation of a health claim, particular attention is given to whether the human studies provided are pertinent to the claim. In this context, the NDA Panel evaluates, among others, whether the human studies use (an) appropriate and well-defined outcome variable(s) of the claimed effect, whether the studies provide evidence from which conclusions can be drawn for the scientific substantiation of the specific claim (e.g. whether efforts have been made to minimise bias), and whether the human studies have been carried out in a study group which is representative of the population group for which the claim is intended (i.e. whether the results obtained in the study population can be extrapolated to the target population).

For human studies which assess outcome variables subject to seasonal variations (e.g. respiratory tract infections, response to allergens), the design of the study should be such that seasonal bias is avoided (e.g. bias introduced by differences between the intervention and control groups regarding the number of subjects investigated in different seasons of the year). The period of enrolment should be defined accordingly.

For studies conducted in non-EU populations, special care should be taken to ensure that intrinsic/extrinsic ethnic characteristics do not influence the physiological response (claimed effect) to the consumption of the food/constituent for which the claim is proposed. Potential confounding factors, such as different dietary habits, should be considered where appropriate. In this respect, it is the responsibility of the applicant to provide a rationale/data which could support the extrapolation of results obtained in non-EU populations to EU populations.

As a general consideration, it is recommended that studies be performed according to scientific standards that are generally accepted by experts in the relevant field, and that they are appropriately reported following, where applicable, EFSA guidelines on statistical reporting[27], or other consensus guidelines published by scientific societies (e.g. CONSORT, STROBE, PRISMA)[28].

The following general considerations regarding the design of human studies submitted for the scientific substantiation of health claims are based on the experience gained by the NDA Panel in the scientific evaluation of health claims related to the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms.

[27] EFSA Guidance on Statistical Reporting: http://www.efsa.europa.eu/en/efsajournal/doc/3908.pdf
[28] Equator network: http://www.equator-network.org/

3.3.1. Human studies assessing self-reported and composite outcome variables

For self-reported outcome variables (e.g. gastro-intestinal symptoms), which are subjective in nature, adequate blinding of subjects and investigators to the intervention is particularly important.

Specific tools, in the form of questionnaires, have been used to measure self-reported outcome variables(s) for claimed effects related to the respiratory and gastro-intestinal tracts in human intervention studies. Considerations on the validation of questionnaires and their use as outcome variables for the scientific substantiation of claims are in Appendix A.

The Panel wishes to highlight that there is no single correct way to demonstrate the validity of a questionnaire. It is a scientific judgement as to the extent to which the information available on validation is sufficient to provide confidence in the validity of the results obtained with the questionnaire for the particular outcome variable(s) under the study conditions. Also, as the appropriateness of a tool will depend on the outcome variable(s) to be measured, the study population, the study design and the study setting, no exhaustive list of acceptable questionnaires can be given.

3.3.2. Extrapolation of results from the study population to the target population

The study population are subjects recruited for human studies, which are submitted for the scientific substantiation of the claim. When the study population (e.g. subjects with a disease) is different from the target group for a claim (e.g. the general population), the suitability of the study population for the scientific substantiation of the claim has to be considered in the context of the specific claim and the target population for which the claim is intended.

Results from studies performed in *non-diseased subjects*, including *subjects at high risk* for a disease which may affect the function targeted by the claim (e.g. subjects with high frequency of urinary tract infections in the previous year for a claim on defence against pathogens in the urinary tract, subjects travelling to third countries for a claim on defence against pathogens in the gastrointestinal tract, subjects performing physical exercise), could be used for the scientific substantiation of health claims.

Subjects with a disease that affects the function mentioned in the claim may be an appropriate study population only in specific cases, e.g. IBS patients for a claim on gastro-intestinal discomfort targeted at the general population (see also section 4.1.1).

Information on the selection and characteristics of the study population in relation to the claimed effect should be provided, particularly when the study population are subjects at high risk for the condition at which the claim is aimed (e.g. ascertainment of infection-free status at baseline in hospitalised subjects for a claim on defence against pathogens). For study subjects under pharmacological treatment(s), evidence for a lack of interaction between the food and the medications used with respect to the claimed effect should also be provided.

The NDA Panel considers on a case by case basis the extent to which it is established that extrapolation from the study population (e.g. subjects with a disease) to the target population (e.g. subjects without the disease) is biologically plausible. In this respect, applicants should provide the rationale or data which could support such extrapolation.

In general, results obtained in infants and young children cannot be used for the scientific substantiation of health claims involving the gastrointestinal tract and/or the immune system, including claims related to (immune) defence against pathogens, for which the target population is adults, and *vice versa*. Evidence or a rationale for extrapolation of the results from a sub-group of the population (study group) to the target population, if the target group is wider or different from the study group, should be provided, and will be considered by the Panel on a case by case basis.

Examples of suitable study populations are considered under specific health claims addressed in the guidance document.

3.4. Evaluation of claims related to essential nutrients compared to non-essential nutrients

Claims proposed for established functions of essential nutrients (vitamins and minerals) are treated differently from claims proposed for functions of non-essential nutrients or other substances. The requirements for the definition of the claimed effect, for the scientific substantiation of the claim, and for establishing conditions of use, differ.

Some vitamins and essential minerals have established roles in physiological processes based on a large body of scientific evidence including deficiency symptoms in humans. For claims for which there is well-established consensus among scientific experts as indicated by authoritative scientific sources as to their substantiation by generally accepted scientific evidence (e.g. many of the functions of essential nutrients), the NDA Panel may rely on such consensus for substantiation of the claim. In such cases it may not be necessary to review the primary scientific studies submitted on the relationship between the food/constituent and the claimed effect. For these claims, conditions of use are set on the basis that any significant amount of the essential nutrient will contribute to the claimed effect (e.g. conditions of use can be linked to nutrition claims).

Claims on the maintenance of (unspecified) functions of the immune system have been evaluated by the NDA Panel with a positive outcome for some essential nutrients[29, 30]. The scientific substantiation of these claims was based on the well-established biochemical role of such nutrients, and/or on deficiency symptoms involving the immune system, rather than on weighing the evidence. The use of unspecified functions of the immune system to substantiate such claims is because symptoms of deficiency of a nutrient can result from effects on multiple physiological functions, and it is sometimes not possible or appropriate to single out a precise function that is affected by deficiency of that nutrient in a particular organ or system (e.g. copper contributes to the normal function of the immune system[31]; vitamin D and contribution to the normal function of the immune system and healthy inflammatory response[32]).

For non-essential nutrients or other substances, claims on the improvement or maintenance of (unspecified) functions of the immune system in general are not sufficiently defined for a scientific evaluation. The specific function of the immune system that is the subject of the claim, together with appropriate outcome variables(s) which may be used for the scientific evaluation of the claimed effect *in vivo* in humans, must be identified, and it is necessary to review the primary studies submitted and to weigh the evidence for the substantiation of these claims. For these claims, conditions of use are set on the basis of the human studies submitted for substantiation by considering the minimum amount of the non-essential nutrient or other substance, which consistently exerts an effect on the function that is mentioned in the claim.

Claims proposed for essential nutrients which do not have an established role on the particular function that the claim mentions (e.g. vitamin C and function of the immune system assessed as reduction of the incidence of common cold during and after extreme physical exercise[33]) will be treated as non-essential for that function. In this context, the particular function of the immune system that the claim is mentioning must be identified, and it is necessary to review the primary studies submitted and to weigh the evidence for the substantiation of these claims.

[29] http://www.efsa.europa.eu/en/efsajournal/doc/1226.pdf
[30] http://www.efsa.europa.eu/en/efsajournal/doc/1229.pdf
[31] http://www.efsa.europa.eu/en/efsajournal/doc/1211.pdf
[32] http://www.efsa.europa.eu/en/efsajournal/doc/1468.pdf
[33] http://www.efsa.europa.eu/en/efsajournal/doc/1226.pdf

4. Function claims

4.1. Claims on gastro-intestinal discomfort

Episodes of abdominal pain or discomfort (e.g. bloating, abdominal pain/cramp, straining and borborygmi [rumbling]), in the absence of organic diseases or biochemical abnormalities, are commonly associated with food or drug intake or with alterations of bowel habits and vary between individuals in frequency and severity.

Symptoms such as abdominal pain, cramp, bloating, straining, borborygmi (rumbling) and sensation of incomplete evacuation are associated with gastro-intestinal discomfort. Reducing gastro-intestinal discomfort is considered an indicator of improved gastro-intestinal function. Reducing gastro-intestinal discomfort is a beneficial physiological effect for the general population.

4.1.1. Claims on gastro-intestinal discomfort in adults

Gastro-intestinal discomfort may be measured by using validated subjective global symptom severity questionnaires, such as described in the consensus opinions[34, 35] (see also EFSA, 2014[36], and Section 3.3.1 of the present guidance document). Changes in one or more of the individual symptoms (e.g. representing different domains of the questionnaire), as well as changes in bowel habits, may be used as supportive evidence for mechanisms by which the food/constituent could exert the claimed effect, but cannot be used alone for the substantiation of a claim on the reduction of gastro-intestinal discomfort. Validated "quality of life questionnaires" may also provide supportive evidence for claims on gastro-intestinal discomfort.

Claims on the reduction of gastrointestinal discomfort have been proposed. The scientific evidence for the substantiation of these claims can be obtained from human intervention studies showing changes in gastro-intestinal discomfort as compared to an appropriate food/constituent which is neutral with respect to the claimed effect. Owing to the fluctuating nature of gastro-intestinal symptoms, evidence for a sustained effect with continuous consumption of the food/constituent over long periods of time (at least 4 to 8 weeks) should be provided[37, 38]. As appropriate outcome variables for this claim are subjective in nature (self-reported), blinding of the intervention is an important consideration when judging the risk of bias of the human studies provided for substantiation (see Section 3.3.1).

With respect to the target population, IBS is a functional bowel disorder characterised by chronic or recurrent abdominal pain or discomfort, mostly associated with defecation abnormalities (consistency and frequency of stools) in the absence of a detectable organic or pathological cause. Episodes of abdominal pain or discomfort occur both in healthy people and in individuals suffering from IBS, and the difference between the two is the higher frequency and/or greater severity of the symptoms in IBS patients. IBS patients or subgroups of IBS patients (Rome III criteria) are generally considered an appropriate study group to substantiate claims on gastro-intestinal discomfort intended for the general population (adults and children).

[34] Veldhuyzen van Zanten SJ, Talley NJ, Bytzer P, Klein KB, Whorwell PJ and Zinsmeister AR, 1999. Design of treatment trials for functional gastrointestinal disorders. Gut, 45 Suppl 2, II69-77.

[35] Irvine EJ, Whitehead WE, Chey WD, Matsueda K, Shaw M, Talley NJ and Veldheuyzen van Zanten SJ, 2006. Design of treatment trials for functional gastrointestinal disorders. Gastroenterology, 130, 1538-1551

[36] http://www.efsa.europa.eu/en/efsajournal/doc/3756.pdf

[37] Irvine EJ, Whitehead WE, Chey WD, Matsueda K, Shaw M, Talley NJ and Veldhuyzen van Zanten SJ, 2006. Design of treatment trials for functional gastrointestinal disorders. Gastroenterology, 130, 1538-1551.

[38] European Medicine Agency (EMA), Committee for Medicinal Products for Human use (CPMP/EWP/785/97 Rev. 1, 25 September 2014): Guideline on the evaluation of medicinal products for the treatment of irritable bowel syndrome. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/09/WC500173457.pdf

4.1.2. Claims on gastro-intestinal discomfort in infants and young children

Claims on gastro-intestinal discomfort have been proposed for infants and young children[39]. Reduction of gastrointestinal discomfort is a beneficial physiological effect for infants and young children.

Unexplained bouts of crying in young infants, traditionally, have been attributed to gastrointestinal disturbances and pain[40]. A specific term, infant colic, is commonly used to reflect this situation in young infants. However, there is no proof that crying in infant colic is caused by pain in the abdomen or any other body part. Infant colic has been included in the list of childhood functional gastrointestinal disorders of the Rome III Coordinating Committee with diagnostic criteria based on infant crying time[41]. Infant pain and discomfort behaviours can also be measured objectively using validated pain scales and infant distress behaviour can be assessed by trained observers using behaviour logs or rating scales, supported by evidence for their validity. The Rome III criteria and validated tools can be used to assess gastrointestinal discomfort in infants once other causes of crying, pain or distress have been excluded. The particular life stage to which the claim applies should be specified.

The scientific evidence for the substantiation of these claims can be obtained from human intervention studies showing changes in gastro-intestinal discomfort (e.g. three weeks) as compared to an appropriate food/constituent which is neutral with respect to the claimed effect.

4.1.3. Claims on the reduction of excessive intestinal gas accumulation

Excessive intestinal gas accumulation generally causes abdominal pain and discomfort. Reduction of excessive intestinal gas accumulation, leading to a reduction in gastrointestinal discomfort, is a beneficial physiological effect[42]. Appropriate outcome variables include, for example, breath hydrogen levels measured by hydrogen breath test, and intestinal gas volume assessed by imaging techniques (e.g. functional magnetic resonance imaging).

4.2. Claims on maintenance of normal defecation

Normal bowel habits vary considerably from person to person with regard to frequency of bowel movements (i.e. number of defecations per interval of time), faecal bulk and consistency of stools. Claims on the maintenance of normal defecation (a bowel function) have been proposed. Maintenance of normal defecation is considered a beneficial physiological effect for the general population.

Constipation is associated with less frequent defecations (e.g. <3 per week), with reduced faecal bulk and harder stools, or both. Constipation leads to gastrointestinal discomfort and may contribute to the development of, for example, diverticular disease. More frequent defecations through, for example, a reduction in transit time, and increased faecal bulk and softer stools, may contribute to the maintenance of normal defecation, provided that they do not result in diarrhoea.

Diarrhoea is characterised by more frequent defecations (e.g. ≥ 3 per day), and is generally accompanied by loose or liquid stools. Diarrhoea may lead to dehydration and gastrointestinal discomfort. In this context, less frequent defecations (e.g. through an increase in transit time and harder stools), may contribute to the maintenance of normal defecation, provided that they do not result in constipation.

[39] http://www.efsa.europa.eu/en/efsajournal/doc/3841.pdf

[40] Shamir R, St James-Roberts I, Di Lorenzo C, Burns AJ, Thapar N, Indrio F, Riezzo G, Raimondi F, Di Mauro A, Francavilla R, Leuchter RH, Darque A, Hüppi PS, Heine RG, Bellaïche M, Levy M, Jung C, Alvarez M and Hovish K, 2013. Infant crying, colic, and gastrointestinal discomfort in early childhood: a review of the evidence and most plausible mechanisms. Journal of Pediatric Gastroenterology and Nutrition, 57 (Suppl. 1), S1-45.

[41] Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF and Taminiau J, 2006. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology, 130, 1519-1526.

[42] http://www.efsa.europa.eu/en/efsajournal/doc/2049.pdf

The scientific evidence for the substantiation of health claims on the maintenance of normal defecation can be obtained from human intervention studies showing an increase in the frequency of defecations and/or a beneficial change in the consistency of stools (lower) and faecal bulk (higher) in subjects with functional constipation at baseline, provided that such changes do not lead to diarrhoea, as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or to no treatment (e.g. control group on usual diet) if duly justified. The scientific evidence for the substantiation of health claims on the maintenance of normal defecation can also be obtained from human intervention studies showing a decrease in the frequency of defecations in subjects with functional diarrhoea at baseline which does not lead to constipation under the same conditions. In this context, beneficial changes in the consistency of stools (higher) and faecal bulk (lower) can be used as supportive evidence for the claim. Evidence for a sustained effect with continuous consumption of the food/constituent over periods of time of at least 4 to 8 weeks should also be provided, owing to the chronic nature of functional constipation/diarrhoea.

Frequency of defecations, stool consistency and faecal bulk can be assessed directly by the investigators or by using validated questionnaires for self-reported outcomes (see Section 3.3.1). Changes in transit time (e.g. by using radio-opaque markers) may be used as supportive evidence for a mechanism by which changes in the frequency of defecations are achieved.

With respect to the study population, results from studies conducted in subjects with functional (chronic) diarrhoea and/or with functional (chronic) constipation, including subjects with IBS, could be used for the scientific substantiation of these claims. However, the rationale for extrapolation of results obtained in subjects with chronic diarrhoea or constipation under pharmacological treatment to the target population for the claim should be provided, and will be considered on a case-by-case basis (e.g. evidence for a lack of interaction between the food and the medications used on the claimed effect).

4.3. Claims on digestion and/or absorption of nutrients

Health claims on improved digestion or absorption of nutrients have been proposed.

4.3.1. Claims on digestion and/or absorption of macronutrients

Whether improved digestion of non-essential nutrients is considered a beneficial physiological effect may depend on the consequences of reduced digestion of that nutrient (e.g. the effect of undigested nutrient in the gastro-intestinal tract).

Claims related to the reduced absorption of non-essential nutrients, such as glucose or cholesterol, are considered in the context of reduced blood concentrations of these nutrients[43, 44].

4.3.1.1. Claims on improved lactose digestion

Lactose maldigestion results from a reduced enzymatic capacity to digest lactose. Individuals with clinical symptoms after lactose intake often display nausea, diarrhoea and symptoms of gastrointestinal discomfort, such us cramping, bloating, and flatulence. Improved lactose digestion may alleviate lactose maldigestion symptoms, and is considered a beneficial physiological effect in individuals with lactose maldigestion[45]. The format of such claims may relate to the effect of a food/constituent (e.g. lactose hydrolysing bacteria or enzymes) on lactose digestion when consumed with lactose containing foods.

[43] Guidance on the scientific requirements for health claims related to appetite ratings, weight management, and blood glucose concentrations: http://www.efsa.europa.eu/en/efsajournal/doc/2604.pdf
[44] Guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health: http://www.efsa.europa.eu/en/efsajournal/doc/2474.pdf
[45] http://www.efsa.europa.eu/en/efsajournal/doc/1763.pdf

To assess lactose digestion, studies in susceptible populations or lactose intolerant subjects, defined either by clinical symptoms or by genotyping for lactase non persistence polymorphism, with appropriate assessment of symptoms of gastrointestinal discomfort, and/or measurement of breath hydrogen and methane, are required.

4.3.2. Claims on digestion and/or absorption of micronutrients

It should be noted that the claimed effect (improved absorption of essential nutrients) is only considered a beneficial physiological effect where absorption is a limiting factor for the maintenance of an adequate status of the nutrient, and where the absorbed nutrient can be utilised by the body. Whether improved absorption of an essential nutrient is considered a beneficial physiological effect may depend of the target population for which the claim is made.

Iron deficiency is one of the most common micronutrient deficiencies in the EU, and can result in anaemia. Non-haem iron is generally not well absorbed in the human intestine, and can be a limiting factor for the maintenance of adequate iron status. Improving iron absorption is considered a beneficial physiological effect. The format of such claims may relate to the effect of a food/constituent (e.g. ascorbic acid) on iron absorption when consumed with iron containing foods[46]. Iron absorption can be measured in humans by generally accepted methods.

Inadequate dietary calcium intake, impaired calcium absorption and low calcium retention may contribute to impaired bone development in early life. The absorption of calcium can be a limiting factor in preterm infants in order to achieve the fetal accretion rate for calcium of 90-120 mg/kg/day[47], in healthy term infants in order to achieve the retention of about 200 mg/day[48], and in infants with disturbances of lipid digestion which can result in insufficient calcium in the body to meet the demands of growing bone. The Panel considers that an increase in calcium absorption leading to an increase in calcium retention is a beneficial physiological effect for infants[49]. Calcium absorption and calcium retention can be measured in humans by generally accepted methods.

4.4. Claims on (immune) defence against pathogens

Defence against pathogens comprises different mechanisms, which act in concert to protect against infection. The presence of pathogenic microorganisms may cause clinical infections at various sites of the body, and defence against pathogens at a specific site of the body is considered a beneficial physiological effect for the general population. For function claims on defence against pathogens, the claim should specify the site of infection (e.g. defence against pathogens in the gastro-intestinal tract, in the upper respiratory tract or in the urinary tract), the type of pathogenic microorganism (e.g. bacteria, virus, fungi), and the target population.

The scientific evidence for the substantiation of health claims related to defence against pathogens 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). The infectious nature of the disease should be established, e.g. by clinical differential diagnosis and/or microbiological data and/or the use of validated questionnaires, depending on the study context and type of infection.

Other outcome variables, such as changes in immune markers, may provide supportive evidence on the biological plausibility and on the mechanism by which the food/constituent could exert the claimed

[46] http://www.efsa.europa.eu/en/efsajournal/doc/1226.pdf
[47] Atkinson SA and Tsang R, 2005. Calcium, magnesium, phosphorus and vitamin D. In: Nutrition of the preterm infant: scientific basis and pratical guidelines. Eds Tsang R, Uauy R, Koletzko B, Zlotkin S. Digital Educational Publishing Inc., Cincinnati, 245-275.
[48] Fomon SJ and Nelson SE, 1993. Calcium, phosphorus, magnesium, and sulfur. In: Nutrition of normal infants. Ed Fomon SJ. Mosby, St. Louis, 192-218.
[49] http://www.efsa.europa.eu/en/efsajournal/doc/2289.pdf

effect (e.g. through the activation of the immune system), but cannot be used alone for the scientific substantiation of these claims.

Vaccination confers immunity to certain infectious diseases. Even if a strict correlation between titres in response to vaccination and protection against infection is not always evident, cut-off values of antibody-titres in response to vaccination indicating protection have been established for many vaccines. Higher responses to vaccination (as measured by increased numbers of individuals attaining protective levels of antibody titres) are appropriate outcome variables for the scientific substantiation of claims related to the immune defence against pathogens.

The (transient) presence of microorganisms and/or their toxins at a particular body site or in the circulation may or may not reflect a clinical infection. In this context, microbiological data could be used instead of (i.e. replace) clinical outcomes related to infections (e.g. incidence, severity and/or duration of symptoms) if evidence is provided that the presence of a particular microorganism (and/or their toxins) at a particular body site, or the presence of a certain amount of the microorganism, would eventually lead to a clinical infection in the target population for which the claim is made (general population or subgroups thereof). The evidence provided will be evaluated by the NDA Panel on a case-by-case basis.

With respect to the study population, subjects without an infection at baseline, including subjects at high risk for infection (e.g. travellers to high risk countries, subjects under heavy physical exercise, elderly individuals in nursing homes, children attending day-care centres, subjects challenged with live viruses/bacteria) could be suitable study groups for the scientific substantiation of claims on (immune) defence against pathogens for the general population, as long as the methods and the inclusion/exclusion criteria used to characterise the study group in relation to the absence of on-going infectious diseases at baseline are clearly defined.

4.4.1. Claims on (immune) defence against pathogens in the gastro-intestinal tract

The presence of pathogenic microorganisms in the gastro-intestinal (GI) tract (e.g. viruses, bacteria, fungi) may lead to the development of GI infections. Maintenance of defence against pathogenic GI microorganisms may protect against the development of GI infections, which is a beneficial physiological effect for the general population.

The scientific evidence for the substantiation of health claims related to defence against pathogens in the GI tract can be obtained from human intervention studies showing an effect on clinical outcomes related to GI infections (e.g. incidence, severity and/or duration of symptoms). For instance, incidence of diarrhoeal episodes may be used as an outcome variable for claims related to defence against pathogens in the gastro-intestinal tract. The infectious aetiology of diarrhoeal episodes should be ascertained. In this context, gastro-intestinal infections 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[50]. Microbiological data could also be used to ascertain the infectious aetiology of diarrhoeal episodes.

4.4.2. Claims on (immune) defence against pathogens in the respiratory tract

Defence against pathogens in the (upper and/or lower) respiratory tract is a beneficial physiological effect for the general population[51].

The scientific evidence for the substantiation of health claims related to defence against pathogens in the respiratory tract can be obtained from human intervention studies showing an effect on clinical

[50] http://www.efsa.europa.eu/en/efsajournal/doc/2167.pdf
[51] http://www.efsa.europa.eu/en/efsajournal/doc/3159.pdf

outcomes related to respiratory infections (e.g. incidence, severity and/or duration of symptoms), either of the upper respiratory tract (such us rhinitis, pharyngitis, sinusitis, otitis media, and common cold), of the lower respiratory tract (such as pneumonia, bronchitis, and bronchiolitis), or both. For instance, upper or lower respiratory tract infections 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 respiratory infection have been applied (i.e. differential diagnosis). Microbiological data could also be used to ascertain the infectious aetiology of clinical episodes.

4.4.3. Claims on defence against pathogens in the urinary tract

Presence of bacteria in the urinary tract may cause symptomatic urinary tract infections (UTIs). UTI is the most common infection in girls and women, with the incidence rising with age and sexual activity. Symptomatic UTIs are usually accompanied by bacteriuria at levels of $\geq 10^5$/mL of midstream urine, and it has been estimated that uropathogenic strains of E. *coli* bacteria are the most common cause of UTIs[52]. Defence against bacterial pathogens in the lower urinary tract is a beneficial physiological effect[53].

The scientific evidence for the substantiation of function claims related to defence against pathogens in the lower urinary tract can be obtained from human intervention studies showing an effect on clinical outcomes related to urinary tract infections (e.g. incidence, severity and/or duration of symptoms).

Bacterial adherence to mucosal surfaces is generally considered an important prerequisite for colonisation and infection with bacteriuria[54]. However, some of the outcome variables proposed for the scientific substantiation of these claims, e.g. *in vitro* inhibition of the bacterial adhesion to uroepithelial cells, are not direct measures of defence against pathogens in the lower urinary tract. Inhibition of the bacterial adhesion to uroepithelial cells *in vitro* does not predict the occurrence of a clinically relevant inhibition of the bacterial adhesion to uroepithelial cells *in vivo* in humans[55, 56]. These outcomes could provide evidence on the biological plausibility and on a mechanism by which a food/constituent provides defence against bacterial pathogens in the lower urinary tract, but they cannot be used in isolation for the scientific substantiation of these claims.

With respect to the study population, subjects without infections of the urinary tract at baseline, but at high risk of infections (e.g. women with past uncomplicated, sporadic or recurrent cystitis), are considered appropriate study groups to substantiate claims on defence against bacterial pathogens in the lower urinary tract for the general population. Where appropriate, the confounding role of medication should be considered.

4.4.4. Claims on defence against vaginal pathogens

Bacterial pathogens (e.g. *Gardnerella vaginalis*) are the most common cause of vaginal infections. Unlike any other anatomical site of the body, most vaginal vaults are dominated by one or more species of *Lactobacillus*. In over 70 % of women, vaginal microbiota is dominated by lactobacilli (>50 %). The diagnosis of bacterial vaginosis (BV) is currently based on the Nugent score. Other pathogenic microorganisms also cause vaginal infections including yeasts (*Candida albicans*) and parasites (*Trichomonas vaginalis*).

[52] Ronald A, 2003. The etiology of urinary tract infection: traditional and emerging pathogens. Dis. Mon. 49, 71-82.
[53] http://www.efsa.europa.eu/en/efsajournal/doc/3656.pdf
[54] Harber MJ and Asscher AW, 1985. Virulence of urinary pathogens. Kidney Int, 28, 717-721.
[55] http://www.efsa.europa.eu/en/efsajournal/doc/3326.pdf
[56] http://www.efsa.europa.eu/en/efsajournal/doc/2215.pdf

Defence against vaginal pathogens is a beneficial physiological effect for the general female population[57]. The claimed effect can be achieved by decreasing the proportion of potentially pathogenic microorganisms in the vagina.

The scientific evidence for the substantiation of function claims related to defence against vaginal pathogens can be obtained from human intervention studies showing a decrease in clinical outcomes related to vaginal infections (e.g. incidence, severity and/or duration of symptoms) and/or a reduction of pathogens following oral consumption of the food/constituent that is the subject of the claim as compared to an appropriate food/constituent which is neutral with respect to the claimed effect, or exceptionally to no treatment (e.g. control group on usual diet). The intra-vaginal route of administration does not provide pertinent data for health claims on food.

With respect to the study population, women without vaginosis at baseline, but at high risk of infections (e.g. women with past uncomplicated, sporadic or recurrent vaginosis), are considered appropriate study groups to substantiate claims on defence against vaginal pathogens for the general population. Where appropriate, the confounding role of medication should be considered.

4.5. Claims on a beneficial change in response to allergens

The general healthy population comprises persons with an increased risk of developing allergic (atopic) reactions, such as allergic rhinitis, allergic asthma, atopic dermatitis and food allergy.

Allergic manifestations, such as asthma, urticaria, eczema, and GI manifestations, are caused by undesirable immune responses to environmental allergens, including food allergens. Beneficial changes in response to allergens may comprise different mechanisms, which act in concert to reduce allergic reactions. The Panel considers that a beneficial change in response to allergens is a beneficial physiological effect for subjects at risk of allergic reactions.

It should be noted that effects of a food on one clinical type of allergy (e.g. respiratory) do not necessarily predict an effect on another type of allergy (e.g. food allergy). The type of allergy that is the subject of the claim should be specified.

The scientific evidence for the substantiation of function claims related to a beneficial change in response to allergens can be obtained from human studies showing a decreased incidence, severity and/or duration of allergic manifestations in subjects at risk of allergic reactions but free of symptoms at baseline. Allergic symptoms are not always easy to distinguish from non-allergic phenomena, and data from self-reported allergies are usually unreliable and insufficient for a diagnosis of allergy. In addition, differences in exposure to the triggering allergen(s) in the intervention and control groups should be carefully considered.

Other outcome variables, such as basophil activation test, tryptase in plasma, and allergen specific IgE, may provide supportive evidence on the (e.g. immune) mechanisms and biological plausibility of a claim related to a beneficial change in response to allergens, but they cannot be used alone for the substantiation of these claims.

5. Disease risk reduction claims

5.1. Claims on the reduction (or beneficial alteration) of a risk factor for infections

The scientific substantiation of health claims on the reduction (or beneficial alteration) of a risk factor for infections 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), together with the reduction (or beneficial alteration) of a risk factor for infections, preferably in the same studies (see Section 3.2.2.2).

[57] http://www.efsa.europa.eu/en/efsajournal/doc/2232.pdf

In this context, evidence for an independent association between the risk factor and the incidence of infections, and for the biological basis through which the risk factor can contribute to the development of infections needs to be provided. Such evidence will be evaluated by the NDA Panel on a case-by-case basis.

The presence of certain microorganisms (or an increase in the number of certain microorganisms) or their toxins at particular sites of the body has been independently associated with an increased risk of infections, and there is evidence for the biological basis through which the risk factor can contribute to the development of infections. Examples include, but are not limited to, the presence of toxigenic *Clostridium difficile* in the GI tract[58], and of uropathogenic *E. coli* strains in the urinary tract[59, 60, 61].

The scientific substantiation of health claims on the reduction (or beneficial alteration) of a well-established risk factor for infections could also be obtained from human intervention studies showing a reduction (or beneficial alteration) of the risk factor. Evidence for an effect on clinical outcomes related to infections (e.g. incidence, severity and/or duration of symptoms) is not required.

For less well established risk factors, additional evidence needs to be provided that a given modification of the risk factor by dietary intervention generally reduces the risk of infections. Such evidence will be evaluated by the NDA Panel on a case-by-case basis.

CONCLUSIONS

This draft guidance document focuses on key issues regarding the substantiation of health claims related to the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms.

The revision takes into account the outcome of a public consultation on a discussion paper together with new scientific evidence available to the NDA Panel and the experience gained to date with the evaluation of health claim applications in the areas of the gastrointestinal tract, the immune system, and defence against pathogenic microorganisms. The guidance document has been structured taking into consideration the comments and the request for clarification received during the public consultation on the discussion paper.

[58] http://www.efsa.europa.eu/en/efsajournal/doc/1903.pdf
[59] http://www.efsa.europa.eu/en/efsajournal/doc/943.pdf
[60] http://www.efsa.europa.eu/en/efsajournal/doc/1421.pdf
[61] http://www.efsa.europa.eu/en/efsajournal/doc/3657.pdf

APPENDIX

Appendix A. Considerations on the validation of questionnaires and their use as outcome variables for the scientific substantiation of claims.

Questionnaires are used to assess subject-reported outcomes, which are subjective in nature. They may assess an outcome at a single time point or longitudinally over time, e.g. changes from baseline. They can be designed to investigate a single concept (e.g. a single symptom) or a combination of concepts (e.g. a combination of symptoms relevant for a specific outcome). Whenever objective measures are available for an outcome they are generally preferred over the use of subjective measures, such as questionnaires. A subjective measurement tool, such as a questionnaire, should have been shown to reliably measure the concept or the combination of concepts it intends to measure. This approach is not different from any new measurement instruments or novel laboratory methods, which have to be validated prior to routine use.

Questionnaires should have been validated (i.e. should meet their pre-determined properties and be suitable for purpose) and should have been shown to be reliable (i.e. ability to yield consistent, reproducible estimates of a true effect), prior to their use in a confirmatory study, for the study population (if the target population is different from the study population, validation for the target population is not needed), in the particular study setting, and the measurement properties of the questionnaire should be known. Validating a questionnaire in the same study in which the questionnaire is used to measure the outcome variable is not appropriate for the purpose of obtaining confirmatory results.

Several criteria have been developed to assess the measurement properties of questionnaires[62, 63] and guidelines on the use of subject-reported outcomes are available[64] and provide guidance on how questionnaires could potentially be validated and on how the most applicable tool for a certain outcome could be selected.

Items which have been recommended to be considered when assessing the validity of a given questionnaire in a specific context are[65]: (1) content validity, (2) internal consistency, (3) criterion validity, (4) construct validity, (5) reproducibility (including agreement and reliability), (6) responsiveness, (7) floor and ceiling effects, and (8) interpretability (see GLOSSARY). These items could be considered by an applicant when determining if a specific questionnaire could be considered appropriate in a given context. The NDA Panel notes that, in some cases, it will not be possible to assess criterion validity in the absence of a gold standard for measuring the intended outcome. However, in cases where such a method is available, criterion validity is an important aspect to consider.

The Panel would like to highlight that particular attention should be paid to the following issues:

- A questionnaire can only be considered to be appropriate if the population in which the questionnaire has been validated is representative of the study population, and if the setting in

[62] Terwee CB, Bot SD, de Boer MR, van der Windt DA, Knol DL, Dekker J, Bouter LM, de Vet HC, 2007. Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol. 60, 34-42. http://www.sciencedirect.com/science/article/pii/S0895435606001740#

[63] Scientific Advisory Committee of the Medial Outcomes Trust, 2002. Assessing health status and quality-of-life instruments: Attributes and review criteria. Quality of Life Research. 11, 193-205. http://rd.springer.com/article/10.1023%2FA%3A1015291021312

[64] U.S. Food and Drug Administration (FDA), 2009. Guidance for Industry. Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims. http://www.fda.gov/downloads/Drugs/Guidances/UCM193282.pdf

[65] Terwee CB, Bot SD, de Boer MR, van der Windt DA, Knol DL, Dekker J, Bouter LM, de Vet HC, 2007. Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol. 60, 34-42. http://www.sciencedirect.com/science/article/pii/S0895435606001740#

843 which the questionnaire has been validated is representative of the setting of the study in
844 which it is to be used.

845 • Any changes made (e.g. modifications of items) to a previously validated questionnaire
846 require a revalidation of the questionnaire.

847 • Validation is language specific and translating a previously validated questionnaire into
848 another language requires further validation steps.

849 • A questionnaire which has been validated for a composite score is not necessarily validated
850 for the individual constructs which make up the composite score and vice versa.

851 • A questionnaire which has been validated to assess an outcome at a single time point may not
852 necessarily be validated to assess changes of an outcome over time (responsiveness).

853 • A questionnaire which has been validated as an interviewer-administered questionnaire may
854 not necessarily be validated in a self-administered setting and vice versa.

855 • A questionnaire which has been validated to assess the severity of a condition may not
856 necessarily be validated to assess the incidence and vice versa.

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858 **GLOSSARY AND ABBREVIATIONS**

AFLP	Amplified fragment length polymorphism
Construct validity	The extent to which scores on a particular instrument relate to other measures in a manner that is consistent with theoretically derived hypotheses concerning the concepts that are being measured.
Content validity	The extent to which the concepts of interest are comprehensively represented by the items in the questionnaire.
Criterion validity	The extent to which scores on a particular instrument relate to a gold standard
E. coli	*Escherichia coli*
Floor and ceiling effects	Lowest or highest possible scores.
GI	Gastro-intestinal
IBS	Irritable bowel syndrome
Internal consistency	A measure of the extent to which items in a questionnaire (sub)scale are correlated (homogeneous), thus measuring the same concept.
Interpretability	The degree to which one can assign qualitative meaning to a quantitative scores.
ITS	Internal Transcribed Spacer
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
RAPD	Randomly amplified polymorphic DNA
Reproducibility	The degree to which repeated measurements in stable persons (test-retest) provide similar answers.
Responsiveness	The ability of a questionnaire to detect clinically important changes over time, even if these changes are small.
RFLP	Restriction fragment length polymorphism analysis
rRNA	Ribosomal RNA
UTI	Urinary tract infection

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