SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 September 2020

doi: 10.2903/j.efsa.2020.6264

Anxiofit-1 and reduction of subthreshold and mild anxiety: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Frank Thies, Sophia Tsabouri, Marco Vinceti, Jean-Louis Bresson and Alfonso Siani

Abstract

Following an application from Anxiofit Ltd., submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Hungary, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Anxiofit-1 and reduction of subthreshold and mild anxiety. The food, Anxiofit-1, *Echinacea angustifolia* root extract, standardised for the content of echinacoside (at least 3%) and the profile of alkamides, which is the subject of the health claim, is sufficiently characterised. The Panel considers that reduction of subthreshold and mild anxiety is a beneficial physiological effect. Subthreshold and mild anxiety are risk factors for anxiety and depressive disorders. One human intervention study showed an effect of Anxiofit-1 (80 mg/day given for 7 days) on the state anxiety and not on the trait subscale in subjects with subthreshold or mild anxiety. These results are supported by two human intervention studies conducted with Anxiofit-1 at 40 mg/day for 7 days and 6 weeks, respectively, which, on their own, cannot be used for the substantiation of the claim either because of methodological limitations or because the results cannot be extrapolated to the target population for the claim. All the human intervention studies submitted have been conducted in a similar setting, the results of the study with Anxiofit-1 given at 80 mg/day have not been confirmed by other research groups. The information submitted by the applicant does not provide evidence for a plausible mechanism by which Anxiofit-1 could exert the claimed effect. The Panel concludes that the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of Anxiofit-1 and reduction of subthreshold and mild anxiety.

Keywords: Anxiofit-1, *Echinacea angustifolia*, anxiety, health claim

Requestor: Competent Authority of Hungary following an application by Anxiofit Ltd.

Question number: EFSA-Q-2020-00032

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

Acknowledgements: EFSA wishes to acknowledge the contribution of WG on Claims: Jean-Louis Bresson, Stefaan de Henauw, Alfonso Siani and Frank Thies to this opinion.

Suggested citation: EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Knutsen HK, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Thies F, Tsabouri S, Vinceti M, Bresson J-L and Siani A, 2020. Scientific Opinion on Anxiofit-1 and reduction of subthreshold and mild anxiety: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA Journal 2020;18(10):6264, 11 pp. https://doi.org/10.2903/j.efsa.2020.6264

ISSN: 1831-4732

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

Table of contents

1. Introduction

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

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

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

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: Anxiofit-1 and reduction of subthreshold and mild anxiety.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of Anxiofit-1, a positive assessment of its safety, nor a decision on whether Anxiofit-1 is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'Anxiofit-1, a food ingredient that contains an *Echinacea angustifolia* hydro-alcoholic root dry extract standardized for the specific alkamide profile'.

Health relationship as claimed by the applicant

According to the applicant, the health effect is related to 'alleviates subthreshold and mild anxiety symptoms'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'the proposed mode of action for these bioactive components in Anxiofit-1 is delivered through the activation of molecular and brain mechanisms involved in anxiety control including binding to CB1/CB2 cannabinoid receptors, inhibition of FAAH enzymes responsible for the degradation of the endocannabinoid anandamide in brain and exerting agonist action on the TRPV (transient receptor potential vanilloid)-1 receptor'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Anxiofit-1 has been shown to ameliorate subthreshold and mild anxiety. Subthreshold and mild anxiety are risk factors in the development of anxiety disorders and depression'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'those having subthreshold and mild anxiety symptoms but who are otherwise healthy'. The quantity of 40–80 mg/day is recommended.

Data provided by the applicant

The health claim application on Anxiofit-1 and reduction of subthreshold and mild anxiety pursuant to Article 14 of Regulation 1924/2006, was presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims (EFSA NDA Panel, 2016a).

As outlined in the General guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

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

The scientific requirements for health claims related to the functions of the nervous system, including psychological functions are outlined in a specific EFSA guidance (EFSA NDA Panel, 2012).

The data claimed as proprietary are: Starting materials – source of stage 1 (plant material) and stage 2 (root dry extract), alkamide fingerprint/profile of Anxiofit-1, alkamide fingerprint/profile of the finished product (i.e. the food supplement in tablet form), the detailed formulation of the food supplement (including the full list of excipients), the specification (acceptance criteria) for the food supplement, information on three unpublished human intervention studies carried out by the applicant, including substantiation of the dose–response relationship, details of individual alkamides within the specific alkamide profile of Anxiofit-1, the manufacturing process of Anxiofit-1 and the manufacturing process of the food supplement containing Anxiofit-1.

The data claimed as confidential are: the results of the stability studies presented, the study protocols and the study reports of the studies submitted and all data that are also claimed proprietary. EFSA has issued its Decision on Confidentiality on 26/5/2020.

3. Assessment

The approach used by the NDA Panel for the evaluation of health claims is explained in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a). In assessing each specific food/health relationship, which forms the basis of a health claim the NDA Panel considers the following key questions:

i) the food/constituent is defined and characterised;
ii) the claimed effect is based on the essentiality of a nutrient; OR the claimed effect is defined and is a beneficial physiological effect for the target population and can be measured *in vivo* in humans;
iii) a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three questions needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of questions (i) and/or (ii) precludes the scientific assessment of question (iii).

3.1. Characterisation of the food/constituent

The food/constituent proposed by the applicant as the subject of the health claim is Anxiofit-1, an *Echinacea angustifolia* root extract. Subspecies of *E. angustifolia* used in the product are *E. angustifolia* DC var. *angustifolia* and *E. angustifolia* DC var. *strigose*. Anxiofit-1 is a hydro-ethanol extract standardised for the content of echinacoside (at least 3%) and the profile of alkamides. The concentration of alkamides and of echinacoside in the final product is measured by high-performance liquid chromatography (HPLC). The alkamide profile of the product was presented in graphic form. The data on the total content of alkamides and amounts of alkamide compounds in several batches of the product were provided (claimed as confidential information).

The applicant provided evidence on bioavailability of alkamides present in *Echinacea* (Matthias et al., 2005; Woelkart et al., 2005, 2008; Guiotto et al., 2008).

Detailed specifications of the manufacturing process, stability information and batch-to-batch variability were provided by the applicant and claimed as confidential information.

The Panel considers that the food Anxiofit-1, an *Echinacea angustifolia* root extract standardised for the content of echinacoside (at least 3%) and the profile of alkamides, which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'alleviates sub-threshold and mild anxiety symptoms'. The proposed target population is 'those having subthreshold and mild anxiety symptoms but who are otherwise healthy'.

As explained in the previous EFSA opinion on the same food/constituent and health effect (EFSA NDA Panel, 2016b), subjects with subthreshold anxiety can be defined as subjects having symptoms of anxiety but not meeting the full diagnostic criteria for anxiety disorders in relation to the number of symptoms and/or their duration. The applicant also specified the criteria used for the definition of mild anxiety: a score $\leq$ 17 in the Hamilton anxiety scale (HAM-A), a score of 8–10 in the hospital anxiety and depression scale – anxiety subscale (HADS-A),or a score of 45–57 points for subscales and 90–114 points for the total score in the state-trait anxiety inventory (STAI). State anxiety reflects the psychological and physiological transient reactions directly related to adverse situations in a specific moment. In contrast, the term trait anxiety refers to a trait of personality, which describes a stable tendency to present state anxiety across many situations.

The applicant proposes that the presence of subthreshold and mild anxiety is a risk factor for the development of anxiety disorders (particularly agoraphobia, generalised anxiety disorder, obsessive compulsive disorder, panic disorder, simple phobia and social phobia) and depressive disorders (particularly major depressive disorder, dysthymia, manic episodes and suicidality as symptoms of bipolar disorders).

As described in the previous EFSA Opinion, to substantiate the relationship between the proposed risk factor and the diseases, the applicant submitted two types of evidence: observational studies showing an association between the proposed risk factor and an increased risk of psychiatric disorders, and intervention studies documenting that a reduction in the risk factor decreases the risk of psychiatric disorders. These studies were mentioned in the previous EFSA opinion (EFSA NDA Panel, 2016a,b).

Upon a request from EFSA to update the evidence on the relationship between the proposed risk factor and the diseases, the applicant provided four additional observational studies on the association between subthreshold and mild anxiety and the above-mentioned diseases. The study populations included the general adult population (Bosman et al., 2019), elderly with visual impairment (Heesterbeek et al., 2017), young adults (Cross et al., 2017), and adolescent girls (Goldstein et al., 2017). In all these studies, subthreshold and/or mild anxiety were associated with an increased risk of either anxiety or depressive disorders.

The Panel considers that reduction of subthreshold and mild anxiety is a beneficial physiological effect. Subthreshold and mild anxiety are risk factors for anxiety and depressive disorders.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in Medline. Keywords used were as follows: "(Echinacea [title/abstract] OR alkamides [title/abstract] OR isobutylamides [title/abstract]) OR alkylamides [title/abstract] AND (anxiety [title/abstract] OR anxiolytic [title/abstract])". Inclusion criteria covered studies in which: (i) subjects were humans; (ii) mild and sub-threshold levels of anxiety were studied; (iii) the time course (trajectory) of consequences or prognosis of mild or sub-threshold anxieties were investigated; and (iv) consequences for anxiety disorders and depression symptoms were evaluated (including suicidality).

An application on Anxiofit-1 and reduction of subthreshold and mild anxiety has previously been evaluated by the NDA Panel with a negative outcome (EFSA NDA Panel, 2016b). The Panel concluded that the scientific evidence was insufficient to establish a cause and effect relationship between the intake of Anxiofit-1 and reduction of subthreshold and mild anxiety.

In the present application, four human intervention studies have been identified by the applicant as pertinent to the health claim, three of which were already evaluated by the Panel in the context of the previous assessment (Haller, 2008, unpublished; Haller et al., 2013; Haller, 2013, unpublished).

The fourth and most recent study submitted with the present application (Haller et al., 2019, unpublished) is a randomised, parallel, two-arm, double-blind, placebo-controlled, single-centre study which investigates the effect of Anxiofit-1 (80 mg/day; two tablets of 20 mg twice daily) given for 7 days vs placebo (identical to the test product containing only excipients) on state and trait anxiety.

Subjects with signs of subthreshold anxiety defined as STAI scores > +1 SD the average of the general population on either the state or the trait anxiety scale (≥ 46 points) were enrolled. Exclusion criteria were any axis I disorder according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV), psychotherapy treatment for anxiety or use of medication for psychiatric conditions.

STAI scores were measured on days 0 and 3 before the intervention, on days 4, 5, 6 and 10 (7-day intervention period) and on days 11, 18, 25 and 32 (3-week follow-up period without intervention). STAI was completed by the participants in the study centre on three occasions at days 0, 3 and 11, and at home for the rest of the measurements. Subjects completing the STAI at the research centre had no additional guidance or support from the staff as STAI is designed as a self-administered questionnaire. Additionally, the Beck Depression Inventory (BDI) and the Perceived Stress Scale (PSS), investigated as exploratory variables, were measured twice, at day 3 and at days 11 (half of the subjects) and 32 (other half of the subjects).

The primary outcome of the study was the STAI scores for the trait and state anxiety scales. Power calculation was based on the results of the study by Haller et al. (2013). Assuming an effect size of 1.21 for state anxiety and of 1.14 for trait anxiety, it was calculated that 43 subjects in total were needed to detect an interaction effect between time and treatment with a power of 95% and a significance level of 5%. The target for recruitment was 64 subjects (32 per group) in order to account for drop-outs.

A coding system of the study products ensured allocation concealment and blinding of subjects and investigators to the intervention.

Compliance was assessed by checking trial diaries, in which subjects were asked to note time of consumption of the study products daily, and by returned leftover tablets. Two subjects in each group did not take one tablet and two subjects in the placebo group omitted two tablets.

Between-group differences were analysed by two-factor analysis of variance (ANOVA), after square-root transformation of data, with factors for time and treatment. Post hoc pair-wise comparisons by the Duncan's test were performed when the interaction between time and treatment was significant. p-Values were Bonferroni corrected. Missing data were imputed using the mean of the group. Missing data concerned the assessments at days 3, 4, 6 and 25 and affected no more than two participants at each time-point.

A total of 64 participants were randomised (mean age 37 ± 2.4 years, 34 women and 30 men). Two subjects were excluded from data analysis (both in the placebo group) because they did not meet the inclusion criteria for the STAI subscales.

Baseline state and trait anxiety scores were comparable between groups at randomisation: the mean (± SE) baseline state anxiety score was 61.2 ± 1.3 in the placebo and 61.0 ± 1.4 in the intervention group. For trait anxiety, values were 49.8 ± 1.8 and 48.1 ± 1.7, respectively.

A significant treatment per time interaction was found for the state anxiety scale of STAI in favour of Anxiofit-1 vs placebo ($p < 0.0001$). STAI state anxiety scores were significantly lower in the Anxiofit-1 group than in the placebo group at the last day of the intervention (median (95% CI) = 53.5 (48.3–54.8) vs 61 (53.6–61.3); $p < 0.05$) and all days of follow-up. No statistically significant differences between Anxiofit-1 and placebo were found for the STAI trait anxiety scale, the BDI or the PSS.

The Panel considers that this study shows an effect of Anxiofit-1 at doses of 80 mg/day taken for seven days on state anxiety as measured by the STAI subscale in subjects with subthreshold and mild anxiety.

The three human intervention studies submitted with the previous application (Haller, 2008, unpublished; Haller et al., 2013; Haller, 2013, unpublished) were reassessed by the Panel together with the additional information and data provided by the applicant upon EFSA's request.

The unpublished study by Haller (2008, unpublished) was a pilot one-arm open-label study assessing the effect of ethyl alcoholic *Echinacea purpurea* root tincture on measures of state and trait anxiety in seven participants. The Panel considers that no conclusions can be drawn from this uncontrolled study, in which a food other than the food which is the subject of this claim was used, for the scientific substantiation of the claim.

In a randomised, parallel, dose-finding study, Haller et al. (2013) investigated the effect of Anxiofit-1 on total STAI scores in a group of volunteers with subthreshold anxiety defined as STAI scores > +1 SD

the average of the general population in each of the subscales (i.e. state anxiety and trait anxiety ≥ 45). Exclusion criteria were any diagnosed axis I disorder according to the DSM-IV and/or medication use for psychiatric conditions in the 6 months prior to the study. Participants were randomly assigned to two groups, using random number tables. They received either one tablet (n = 15, 10 females) or two tablets (n = 17, 11 females) per day containing 20 mg of Anxiofit-1 (i.e. 20 and 40 mg/day, respectively) for a period of 7 days (days 4–10 of the study). STAI scores, using a structured self-assessment diary technique, were obtained at baseline (days 1 and 3); on days 5, 6, 10, and on days 17 and 24 during the 2-week follow-up period. At the same time points, subjects were also asked to note any deviations from the protocol in the diaries. The Panel notes that this study was not placebo-controlled. Upon a request from EFSA, the applicant clarified that the study was not blinded neither for the participants nor for the investigators.

Power was calculated based on an assumed decrease of 20 points in total STAI scores after the intervention. The assumed SD for power calculation was not reported, nor was this information provided by the applicant upon request from EFSA. It is stated that 16 subjects per group were calculated for a power of 95% at a significance level of 5%. Accounting for drop-outs, 40 subjects were planned to be randomised. This recruitment target was not met (because the pace of recruitment was slower than expected) and results are presented for 33 subjects (22 women, 11 men; mean age 40.6 ± 13.2 years). Upon a request from EFSA, the applicant clarified that no major protocol violations occurred, that compliance was full, that all randomised participants finished the study and the calculated sample of 32 subjects was reached when recruitment was stopped.

The effect of Anxiofit-1 on STAI scores was assessed by two-way ANOVAs with treatment and time as factors.

A significant treatment by time interaction ($p < 0.0002$) for total STAI scores was reported. STAI scores for the 20 mg/day group showed no significant changes during the study, but scores for the 40 mg/day group significantly decreased by day 6 compared to baseline and remained significantly lower for the remainder of the study (all comparisons were Bonferroni corrected). The Panel notes that the baseline values used in the statistical analysis were taken at the screening visit and not at the randomization visit. This decision was not justified by the applicant.

Pairwise comparisons between the groups (data provided by the applicant upon request from EFSA) showed that both state anxiety scores (mean ± SEM: 20 mg/day: 60.60 ± 1.20 vs 40 mg/day: 50.20 ± 2.14, $p = 0.013$) and trait anxiety scores (20 mg/day: 59.47 ± 1.08 vs 40 mg/day: 50.88 ± 1.76, $p = 0.025$) were significantly lower in the 40 mg/day group at the end of the intervention period (day 10). Total STAI scores were also significantly lower in the 40 mg/day group on days 6 (i.e. 20 mg/day: 120.87 ± 1.85 vs 40 mg/day: 105.65 ± 4.17, $p = 0.040$) and 10 (i.e. 20 mg/day: 120.07 ± 2.12 vs 40 mg/day: 101.12 ± 3.68, $p = 0.009$) of the study. All comparisons underwent Bonferroni correction.

The Panel considers that this study provides some evidence that Anxiofit-1 (40 mg/day) may decrease anxiety measured by STAI (state anxiety, trait anxiety and total anxiety) in subjects with subthreshold and mild anxiety. However, the Panel notes that lack of blinding and lack of a placebo group are methodological limitations for self-reported outcomes and considers that the results of this study cannot be used on their own for the scientific substantiation of the claim.

The same group of investigators (Haller, 2013, unpublished) studied the effect of Anxiofit-1 in a group of patients with generalised anxiety disorder (GAD) in a randomised, two-arm, placebo-controlled, double-blind, five-centre study. Patients with GAD diagnosed according to DSM-IV criteria, with a HAM-A score between 17 and 25 points at the screening and randomisation visits (indicating mild to moderate anxiety), and a total BDI score < 10 (indicating minimal depression) were recruited. Exclusion criteria were any axis I or axis II disorder according to DSM-IV, serious suicidal risk, receiving psychotherapy or on medication for psychiatric conditions or on medication/supplements/foods with psychoactive properties. The Panel notes that the study population were patients with a diagnosis of GAD according to DSM-IV criteria, and not the target population for the claim. The number of patients recruited at each centre varied from one to nine.

Three days after the recruitment visit, patients were randomised to consume either Anxiofit-1 (40 mg/day; 2 × 20 mg) or an identical placebo for 6 weeks using a central randomisation system and stratification of participants by minimisation using HAM-A scores as criterion (< or > 20). Upon a request from EFSA, the applicant clarified that stratification and assignment of the subjects to one of the two intervention arms was performed centrally for all centres according to the blinded randomisation list produced by the study statistician and the numbers were assigned to the participants in chronological order.

The sample size of 24 participants (12 subjects per group) was estimated based on the results of HAM-A and on general considerations about feasibility and precision around the mean as described by Julious (2005) and van Belle (2002). The applicant argued that this approach was chosen because there were no prior studies conducted in subjects with GAD.

The primary outcome of the study, as reported in the Statistical Analysis Plan (SAP), was scores on the HAM-A scale. It was measured on four occasions (screening, randomisation, and days 14 and 42). HADS-A was measured on eight occasions (screening, randomisation, and days 2, 7, 14, 16, 28 and 42) as one of the secondary outcomes of the study. The Panel notes that the HAM-A scale is considered to be an inadequate outcome measure because it poorly discriminates between generalised anxiety disorder and depression (Koerner et al., 2010) and these results will therefore not be further discussed in this opinion. Depression symptoms and life events were assessed by the BDI and the Perceived Stress Scale (PSS). Compliance was assessed by returned tablets and it was reported as full (100%).

Two-factor ANOVA was used in the statistical analysis of score differences from baseline with factors for time and treatment. The Newman–Keuls test was used for post hoc comparisons.

A total of 26 subjects were randomised (age 24–59 years, 19 females and 7 males, n per group not reported). Two participants dropped out (one in the test group and one in the placebo group). According to the SAP, analyses in the per-protocol (PP) population and in the full analyses set were planned. In the unpublished report submitted to EFSA, the results were reported for the 24 participants (73% women, mean age 43.2 years) who completed the study.

HADS-A scores at the randomisation visit were (mean ± SE) 11.8 ± 0.8 and 9.5 ± 0.4 in the intervention and control groups, respectively. In the repeated measures analysis of changes from baseline in HADS-A scores, there were significant main effects of time ($p = 0.0001$) and treatment ($p = 0.00002$). The interaction between time and treatment in the two-factor analysis was not statistically significant. There were statistically significant differences between groups at day 16 (control (mean ± SE): –2.6 ± 0.6 vs intervention: -4.8 ± 0.8; read from graph; $p = 0.03$) and day 28 (–3.1 ± 0.8 vs –6.1 ± 0.8, $p = 0.02$), but not at the other visits, including day 42 at the end of the study (–4.4 ± 0.8 vs –5.6 ± 0.8).

The Panel notes that no treatment per time interaction was observed in the two-factor analysis. Statistically significant differences were only observed at intermediate time points, but not at the final visit nor between groups over time (as indicated by the lack of a significant treatment per time interaction).

The Panel considers that this study provides limited evidence for a transient effect of Anxiofit-1 on anxiety in patients with GAD. The results of this study cannot be extrapolated to the target population of the claim but could be used as supportive evidence for the scientific substantiation.

The Panel considers that one human intervention study (Haller et al., 2019, unpublished) shows an effect of Anxiofit-1 (80 mg/day given for 7 days) on the state anxiety and not on the trait subscale in subjects with subthreshold or mild anxiety. These results are supported by two human intervention studies conducted with Anxiofit-1 at 40 mg/day (Haller et al., 2013, 2013 unpublished) for 7 days and 6 weeks, respectively, which, on their own, cannot be used for the substantiation of the claim either because of methodological limitations or because the results cannot be extrapolated to the target population for the claim. The Panel also notes that all the human intervention studies submitted have been conducted in a similar setting, and that the results of the study by Haller et al. (2019, unpublished) have not been confirmed by other research groups.

Mechanism of action proposed

The applicant claimed that alkamides can act as cannabinomimetics at both the cannabinoid CB1 and CB2 receptors and can also inhibit the anandamide-degrading enzyme fatty acid amid hydrolase (FAAH). The applicant also proposed that some *Echinacea* alkamides inhibit the enzyme FAAH that degrades the endocannabinoid anandamide in the brain, which may increase endocannabinoid signalling. The applicant also suggested that as yet unidentified constituents of *Echinacea* extracts may activate TRPV1 receptors, which are involved in peripheral pain reception and probably in the regulation of affective behaviours. The applicant noted the role of the hippocampus in anxiety, and presented a study showing that *Echinacea angustifolia* root extracts regulate excitatory, but not inhibitory, synaptic transmission of rat hippocampal neurones which might explain the anxiolytic effects.

The Panel notes that the evidence for a mechanism by which Anxiofit-1 could exert the claimed effect was already assessed by the Panel in the previous opinion on Anxiofit-1 (EFSA NDA Panel,

2016a,b). No additional evidence for the proposed mechanism of action was presented with the current application.

The Panel considers that the evidence provided by the applicant for the mechanisms by which Anxiofit-1 could exert an effect on anxiety is mostly speculative. The Panel considers that no evidence has been provided by the applicant for a plausible mechanism by which Anxiofit-1 could exert the claimed effect *in vivo* in humans.

Weighing the evidence

In weighing the evidence, the Panel took into account that one human intervention study showed an effect of Anxiofit-1 (80 mg/day given for 7 days) on the state anxiety and not on the trait subscale in subjects with subthreshold or mild anxiety. These results are supported by two human intervention studies conducted with Anxiofit-1 at 40 mg/day for 7 days and 6 weeks, respectively, which, on their own, cannot be used for the substantiation of the claim either because of methodological limitations or because the results cannot be extrapolated to the target population for the claim. The Panel also notes that all the human intervention studies submitted have been conducted in a similar setting, that the results of the study with Anxiofit-1 given at 80 mg/day have not been confirmed by other research groups, and that the information submitted by the applicant does not provide evidence for the proposed mechanism by which Anxiofit-1 could exert the claimed effect.

The Panel concludes that the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of Anxiofit-1 and reduction of subthreshold and mild anxiety.

4. Conclusions

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

- the food/constituent, Anxiofit-1, *Echinacea angustifolia* root extract standardised for the content of echinacoside (at least 3%) and the profile of alkamides, which is the subject of the health claim, is sufficiently characterised.
- the claimed effect proposed by the applicant is 'alleviates subthreshold and mild anxiety symptoms'. The target population proposed by the applicant is 'those having subthreshold and mild anxiety symptoms but who are otherwise healthy'. Reduction of subthreshold and mild anxiety is a beneficial physiological effect. Subthreshold and mild anxiety is a risk factor for anxiety and depressive disorders.
- the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of Anxiofit-1 and reduction of subthreshold and mild anxiety.

Documentation as provided to EFSA

Health claim application on Anxiofit-1 and reduction of subthreshold and mild anxiety pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0493_HU). Submitted by Anxiofit Ltd., 18 Nyul Street, Budapest, 1026 Hungary.

Steps taken by EFSA

1) This application was received by EFSA on 10/01/2020.
2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
3) The scientific evaluation procedure started on 26/02/2020.
4) On 27/02/2020, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 12/03/2020 and was restarted on 27/03/2020, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
5) On 15/06/2020, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 7/07/2020 and was restarted on 22/07/2020, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
6) During its meeting on 23/09/2020, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to the consumption of Anxiofit-1 and reduction of subthreshold and mild anxiety.

References

Bosman RC, Ten Have M, de Graaf R, Muntingh AD, van Balkom AJ and Batelaan NM, 2019. Prevalence and course of subthreshold anxiety disorder in the general population: a three-year follow-up study. Journal of Affective Disorder, 247, 105–113.

Cross SPM, Scott J and Hickie IB, 2017. Predicting early transition from sub-syndromal presentations to major mental disorders. British Journal of Psychiatry Open, 3, 223–227.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012. Guidance on the scientific requirements for health claims related to functions of the nervous system, including psychological functions. EFSA Journal 2012;10(7):2816, 13 pp. https://doi.org/10.2903/j.efsa.2012.2816

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

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016b. Scientific opinion on Anxiofit-1 and reduction of subthreshold and mild anxiety: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA Journal 2016;14(1):4365, 13 pp. https://doi.org/10.2903/j.efsa.2016.4365

Goldstein BL, Kotov R, Perlman G, Watson D and Klein DN, 2017. Trait and facet-level predictors of first-onset depressive and anxiety disorders in a community sample of adolescent girls. Psychological Medicine, 48, 1282–1290.

Guiotto P, Woelkart K, Grabnar I, Voinovich D, Perissutti B, Invernizzi S, Granzotto M and Bauer R, 2008. Pharmacokinetics and immunomodulatory effects of phytotherapeutic lozenges (bonbons) with Echinacea purpurea extract. Phytomedicine, 15, 547–554.

Haller J, 2008. The effects of *Echinacea* preparations on anxiety – a pilot study (unpublished study report).

Haller J, 2013. The effects of Anxiofit-1® in generalized anxiety (unpublished study report).

Haller J, Freund TF, Pelczar KG, Furedi J, Krecsak L and Zambori J, 2013. The anxiolytic potential and psychotropic side effects of an *Echinacea* preparation in laboratory animals and healthy volunteers. Phytotherapy Research, 27, 54–61.

Haller J, Krecsak L and Zámbori J, 2019. Double-blind placebo controlled study of the anxiolytic effects of a standardized *Echinacea* extract (unpublished study report).

Heesterbeek TJ, van der Aa HPA, van Rens GHMB, Twisk JWR and van Nispen RMA, 2017. The incidence and predictors of depressive and anxiety symptoms in older adults with vision impairment: a longitudinal prospective cohort study. Ophthalmic and Physiological Optics, 37, 385–398.

Julious SA, 2005. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceutical Statistics, 4, 287–291.

Koerner N, Antony MM and Dugas MJ, 2010. Limitations of the Hamilton Anxiety Rating Scale as a primary outcome measure in randomized, controlled trials of treatments for generalized anxiety disorder. The American Journal Of Psychiatry, 167, 103–104.

Matthias A, Addison RS, Penman KG, Dickinson RG, Bone KM and Lehmann RP, 2005. *Echinacea* alkamide disposition and pharmacokinetics in humans after tablet ingestion. Life Sciences, 77, 2018–2029.

Van Belle G, 2002. Statistical rules of thumb, 2nd Edition. John Wiley & Sons Inc, ISBN-13. pp. 978–0470144480.

Woelkart K, Koidl C, Grisold A, Gangemi JD, Turner RB, Marth E and Bauer R, 2005. Bioavailability and pharmacokinetics of alkamides from the roots of *Echinacea angustifolia* in humans. Journal of Clinical Pharmacology, 45, 683–689.

Woelkart K, Dittrich P, Beubler E, Pinl F, Schoop R, Suter A and Bauer R, 2008. Pharmacokinetics of the main alkamides after administration of three different *Echinacea purpurea* preparations in humans. Planta Medica, 74, 651–656.

Abbreviations

ANOVA	analysis of variance
BDI	Beck depression inventory
CB	cannabinoid
DSM	diagnostic and statistical manual of mental disorders
FAAH	fatty acid amide hydrolase
GAD	generalised anxiety disorder
HADS-A	hospital anxiety and depression scale – anxiety subscale
HAM-A	Hamilton anxiety inventory
HPLC	high-performance liquid chromatography
NDA	Nutrition, Novel Foods and Food Allergens
PP	per protocol
SAP	statistical analysis plan
STAI	state-trait anxiety inventory
PSS	perceived stress scale
TRPV	transient receptor potential vanilloid