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Symbiosal® and lowering of blood pressure and reduced risk of hypertension: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006

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Abstract

Following an application from Han-Biotech GmbH, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Symbiosal®, lowering of blood pressure and reduced risk of hypertension. The Panel considers that the food, Symbiosal®, which is the subject of the health claim, and the food, table salt, which Symbiosal® should replace, are sufficiently characterised. Lowering of blood pressure is a beneficial physiological effect. Increased blood pressure is a risk factor for hypertension. In weighing the evidence, the Panel took into account that one human study with some methodological limitations showed an effect of Symbiosal® on blood pressure in the context of a self-selected diet with a maximum of 3 g/day added salt. The Panel also took into account that no other human studies in which these results have been replicated were provided, that the animal studies did not support the results of the human study, that no evidence was provided in support of a mechanism by which Symbiosal® could induce a decrease in blood pressure upon oral consumption as compared to table salt *in vivo* in humans, and the low biological plausibility of the effect observed in the human intervention study. The Panel concludes that a cause and effect relationship has not been established between the consumption of Symbiosal® and lowering of blood pressure.

Keywords: Symbiosal®, chitosan, blood pressure, hypertension, health claim

Requestor: Competent Authority of Germany following an application by Han-Biotech GmbH

Question number: EFSA-Q-2018-00002

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Summary

Following an application from Han-Biotech GmbH, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Germany, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to Symbiosal®, lowering of blood pressure and reduced risk of hypertension.

The scope of the application was proposed to fall under a health claim referring to disease risk reduction. The application included a request for the protection of proprietary data.

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications and the EFSA guidance on the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health.

The food that is the subject of the health claim is Symbiosal®, which, according to the applicant, should replace table salt (i.e. sodium chloride, NaCl) in order to obtain the claimed effect. NaCl is a well characterised salt. Symbiosal® is produced by mixing sea salt (97%) with chitosan (3%), following a patented manufacturing process. Chitosan is a linear cationic polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and *N*-acetyl-*D*-glucosamine. The Panel considers that the food, Symbiosal®, which is the subject of the health claim, and the food, table salt (i.e. NaCl), which Symbiosal® should replace, are sufficiently characterised.

The claimed effect proposed by the applicant is 'lowers the rising of blood pressure when used as a replacement of traditional table salt. The rising of blood pressure is a risk factor for hypertension'. The target population proposed by the applicant is 'healthy mild hypertensive subjects, and people who want/need to lower the rising of their blood pressure'. The Panel considers that lowering of blood pressure is a beneficial physiological effect. Increased blood pressure is a risk factor for hypertension.

The applicant submitted four human studies. The Panel considers that the one human study with methodological limitations from which conclusions can be drawn shows a decrease in systolic blood pressure (SBP) and diastolic blood pressure (DBP) when 3 g Symbiosal® per day was consumed instead of added table salt for 8 weeks. The Panel considers that the animal studies do not support the results of the human study. The Panel considers that no evidence has been provided in support of a mechanism by which Symbiosal® could induce a decrease in BP upon oral consumption as compared to table salt *in vivo* in humans.

In weighing the evidence, the Panel took into account that one human study with some methodological limitations showed an effect of Symbiosal® on BP as compared to table salt when consumed in doses up to 3 g/day for 8 weeks in the context of a self-selected diet. The Panel notes that the mean difference in SBP changes between the Symbiosal® and the table salt groups was about 10 mmHg. The Panel also took into account that no other human studies were provided in which these results have been replicated, and that the animal studies did not support the results of the human study. Furthermore, no evidence was provided to suggest a mechanism whereby Symbiosal® could induce a decrease in BP upon oral consumption as compared to table salt *in vivo* in humans. In this context, the Panel notes the low biological plausibility for the effect reported in the human intervention study.

On the basis of the data provided, the Panel concludes that a cause and effect relationship has not been established between the consumption of Symbiosal® and lowering of blood pressure.

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1. Introduction

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

Regulation (EC) No 1924/2006[1] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 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 of this Regulation, an application for 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 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: Symbiosal® and lowering of blood pressure and reduced risk of hypertension.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of Symbiosal®, a positive assessment of its safety, nor a decision on whether Symbiosal® 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 17 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 constituent that is the subject of the claim is Symbiosal®, which is a food supplement containing sea salt (97%) plus chitosan (3%).

Health relationship as claimed by the applicant

According to the applicant, consumption of Symbiosal® instead of standard table salt lowers blood pressure, thereby lowering the risk of hypertension. The applicant indicated 'rising of blood pressure' as the risk factor and 'high blood pressure (hypertension)' as the disease.

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

With regard to the mechanism by which the food would exert the claimed effect, the applicant states that the mechanism is not clearly known but some findings suggest that chitosan may inhibit the increase of angiotensin-converting enzyme (ACE) induced by acute salt intake. The applicant also claimed that this inhibition might be mediated by blocking or counteracting the hypertensive effect of the chloride ions in sodium chloride (NaCl).

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Symbiosal® has been shown to lower the rising of blood pressure when used as a replacement of traditional table salt. The rising of blood pressure is a risk factor for high blood pressure (hypertension)'.

[1] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

Specific conditions of use as proposed by the applicant

The applicant has proposed to replace standard table salt in the diet by Symbiosal®, up to a maximum total added salt intake of 3 g per day. This salt replacement (and restriction to the maximum of 3 g per day) should be accompanied by other lifestyle improvements, such as eating less fat and sugar and increasing physical exercise.

The target populations proposed by the applicant are subjects with prehypertension (SBP 130–139 mmHg and/or DBP 80–89 mmHg), and subjects presenting with recently discovered mild to moderate hypertension. Children, pregnant women and lactating women should not consume Symbiosal®.

Data provided by the applicant

Health claim application on Symbiosal® and lowering of blood pressure pursuant to Article 14 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.[2]

As outlined in the General guidance for stakeholders on health claim applications,[3] it is the responsibility of the applicant to provide all the available evidence.

This health claim application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006 in relation to three studies by Allaert presented in five documents (2013a,b, 2015, 2017a,b).

The application does include a request for data confidentiality in relation to manufacturing process.

2.2. Methodologies

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

The scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health are outlined in a specific EFSA guidance (EFSA NDA Panel, 2018).

3. Assessment

3.1. Characterisation of the food/constituent

The food proposed by the applicant as the subject of the health claim is Symbiosal® which, according to the applicant, should replace table salt (i.e. NaCl) in order to obtain the claimed effect (i.e. lowering of blood pressure).

NaCl is a well characterised salt consisting of sodium and chloride in a 1:1 molar ratio.

It is well established that high sodium intakes, mainly as NaCl (table salt), increase blood pressure, and that reducing dietary NaCl intakes helps to maintain a normal blood pressure (EFSA NDA Panel, 2011).

Symbiosal® is produced by mixing sea salt (97%) with chitosan (3%), following a patented manufacturing process. Chitosan is a linear cationic polysaccharide produced by partial deacetylation of chitin derived from crab shells and composed of randomly distributed β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine.

An overview of the manufacturing process of Symbiosal® and information regarding its stability were provided.

The Panel considers that the food, Symbiosal®, which is the subject of the health claim, and the food, table salt (i.e. NaCl), which Symbiosal® should replace, are sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'lowers the rising of blood pressure when used as a replacement of traditional table salt. The rising of blood pressure is a risk factor for hypertension'. The target population proposed by the applicant is 'subjects presenting prehypertension (SBP 130 to

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

[3] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. 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

139 mmHg and/or DBP 80 to 89 mmHg), and subjects presenting recently discovered mild to moderate hypertension'.

Blood pressure is the pressure (force per unit area) exerted by circulating blood on the walls of blood vessels. Hypertension, defined by convention as blood pressure above 140 mmHg (systolic) and/or 90 mmHg (diastolic), may compromise normal arterial and cardiac function.

The Panel considers that lowering of blood pressure is a beneficial physiological effect. Increased blood pressure is a risk factor for hypertension.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in Medline, Cochrane Library, Science Direct, Scirus, Springerlink and Wiley Interscience using the search terms 'Symbiosal®', 'salt', 'NaCl', 'chitosan', 'blood pressure', 'hypertension', 'systolic' and 'supplementation'. Publications in English, French and Spanish were included. In addition, reference lists of retrieved articles were searched manually to identify relevant studies. Trials were included if they were 'well designed' (i.e. randomised and blinded, with an adequate number of subjects). Studies in subjects on blood pressure lowering medication were excluded. Manual searches were also performed.

The Panel has issued an opinion on a health claim related to Symbiosal® and lowering of blood pressure and reduced risk of hypertension pursuant to Article 14 of Regulation (EC) No 1924/2006 with an unfavourable outcome (EFSA NDA Panel, 2015). In weighing the evidence, the Panel took into account that one human study (Allaert, 2013a,b) with methodological limitations showed a decrease in blood pressure when Symbiosal® was consumed instead of table salt for 8 weeks. The Panel also took into account that no other human studies in which these results have been replicated were provided, that the animal studies did not support the results of the human study, and that no evidence was provided for a mechanism by which Symbiosal® could exert the claimed effect.

Human intervention studies

The applicant submitted the human intervention study which was already assessed by the Panel in the previous opinion (Allaert, 2013a,b) and three other human studies described in four publications (Kato et al., 1994; Allaert, 2015, 2017a,b) as being pertinent to the claim. The human intervention study submitted with the previous application (Allaert, 2013a,b) was reassessed by the Panel based on the experience gained in the evaluation of health claim applications since the publication of the previous opinion (EFSA NDA Panel, 2015).

The study was randomised, double-blind, controlled, two-period, cross-over study, which investigated the effect of replacing 3 g/day of table salt by 3 g of Symbiosal® on blood pressure in 40 adult outpatients with SBP 140–159 mmHg or DBP 90–99 mmHg who had never received antihypertensive medications. There was no run-in period prior to the intervention and the two cross-over periods lasted 8 weeks each, separated by a washout of 2 weeks.

Power calculations indicated that, considering a standard deviation of 15 mmHg, 30 subjects would yield a power of 90% (for $\alpha = 0.05$) to detect a difference in SBP of 10 mmHg between Symbiosal® and table salt (Allaert, 2013b, unpublished study report). Considering a 25% drop-out rate, 40 participants were recruited and all were randomised. Subjects were recruited by 13 primary care physicians, with one to four patients recruited per physician.

Participants were randomised with respect to the order in which they received Symbiosal® or regular table salt in blocks of 8 according to a computer-generated code. Subjects were randomised sequentially from 1 to 40 as they were recruited for the study. Each physician received 8 sequentially numbered product kits. Participants were instructed to follow lifestyle advice about physical exercise and nutrition (e.g. to eat less fat and less sugar) and to restrict their added salt intake to a maximum of 3 g/day. They were asked to use the saltcellars they were given containing either Symbiosal® or table salt. Compliance was assessed by weighting the saltcellars at the end of each cross-over period. The Panel notes that there was no attempt to control dietary salt intake other than by dietary advice.

Blood pressure was measured at week 0 (i.e. baseline), week 8 (i.e. end of the first intervention period), week 10 (i.e. end of the washout period) and week 18 (i.e. end of the second intervention period) by a medical practitioner using an approved electronic measuring device with study participants in a sitting position, after a 10-min rest, and at three different time points separated by 3-min intervals. The average of the three measurements was used.

A total of 40 individuals (mean age 58.6 ± 12 years; mean BMI 26.4 ± 4.9 kg/m^2; 24 women) were randomised. Two subjects dropped out of the study (reasons reported) and one omitted the washout period.

Two-way analysis of variance (ANOVA) was used in the statistical analysis of the data. Where no significant carry-over effect was found, the model was simplified by removing this effect. When the ANOVA indicated an overall treatment effect (i.e. $p < 0.05$), comparisons between the means of the two interventions (i.e. Symbiosal® or NaCl) were performed using a two-sided paired t-test. The statistical analysis was conducted using intention-to-treat (ITT, n = 40, all randomised participants), with the last observation carried forward for missing data and 'per protocol' (PP, n = 37, subjects with complete data sets).

Significant period effects were observed for SBP ($p = 0.0006$) and DBP ($p < 0.0001$), i.e. the highest reductions in blood pressure were observed during the first cross-over period (mean decrease in SBP by 9.9 ± 10.0 mmHg for the 1st period and 1.6 ± 8.4 mmHg for the 2nd period, and mean decrease in DBP by 8.9 ± 7.7 mmHg for the 1st period and 1.5 ± 5.6 mmHg for the 2nd period). This indicates that the treatment effect depends upon the order in which Symbiosal® and regular table salt were received, which does not allow the drawing of conclusions on the effect of Symbiosal® on blood pressure. The Panel therefore considers that no conclusions can be drawn from this cross-over study for the scientific substantiation of the claim.

The new study provided by the applicant (Allaert, 2017a) was a randomised, two-arm, parallel, double-blind study which investigated the effects of consuming Symbiosal® for 8 weeks on blood pressure as compared to regular table salt in a group of adult outpatients (aged 18–70 years) with SBP between 130 and 139 mmHg. Upon a request from EFSA, the applicant also submitted unpublished study report (Allaert, 2017b).

Antihypertensive treatment, allergic reactions to seafood and having a history of heart failure, coronary artery disease, stroke or transient ischaemic attack, peripheral arterial disease, kidney failure or other chronic kidney disease, and diabetes were all exclusion criteria. The Panel notes that the process of enrolment was not well described in the publication or the study report. Subjects received a saltcellar containing Symbiosal® or table salt. Participants were instructed not to use more that 3 g of salt per day. The applicant claimed that both products were visually identical.

Participants underwent a run-in period of 1 week, followed by the intervention lasting 8 weeks, and attended three visits at the clinical centre: a screening visit, an inclusion visit (baseline), and a final visit.

At each visit, BP was measured using an electronic device. BP was measured in the lying position three times separated by at least 3 min after a 10-min initial rest. BP values were calculated as the average of the three measurements. Participants were also equipped with the BP monitor and measured BP at home (at 7 p.m. every day during run-in period and the final week of the intervention period, and three times a week from weeks 1 to 7).

The primary outcome of the study was the difference in SBP changes between the Symbiosal® and the table salt groups during intervention measured by the investigators. The secondary outcomes were changes in SBP measured by participants during the intervention period between groups, the proportion of subjects with SBP under 130 mmHg at the end of the study, changes in DBP, blood and urinary sodium, potassium and chloride, and the presence of adverse events. The Panel notes that there was no attempt to control dietary salt intake other than by dietary advice.

Sample size was calculated assuming a 10-mmHg difference in SBP change between groups with a SD of 10 mmHg, $\alpha = 0.05$ and a power of 80%. It was calculated that 17 participants had to be included in each arm of the study. A total of 21 participants per arm were targeted, assuming a 15% dropout rate. Block randomisation with the PROC plan of the SAS software was used. No stratification based on age, sex or other characteristics was performed. The Panel notes that baseline BP values were not taken into consideration in the statistical analysis.

Between-group differences in SBP and DBP during the intervention were assessed by repeated measures ANOVA. Differences in the percentage of participants with a SBP lower than 130 mmHg at the end of the study were assessed using the chi-square test. A total of 58 subjects were enrolled at the screening visit and 41 (20 women, mean age 51.0 ± 16.0 years) were randomised at the inclusion visit (Symbiosal® n = 22; table salt n = 19).

All participants finished the study with no major protocol violation. However, the Panel notes that two participants were older than 70 years and that two other participants had SBP at randomisation outside the limits established as inclusion criteria. These deviations from the protocol were considered as 'minor' by the investigator and these subjects were not excluded from data analysis. The results

were presented for all 41 participants. For the purpose of this opinion, only results related to blood pressure, urinary sodium excretion and body weight are described.

Mean SBP and DBP at baseline were not significantly different between groups. SBP measured by the investigators (the main outcome of the study) decreased by 7.7 ± 5.9 mmHg in the Symbiosal® group (from 133.8 ± 5.7 to 126.1 ± 6.5 mmHg) and increased by 3.7 ± 6.0 mmHg in the control group (from 136.6 ± 10.3 to 140.4 ± 8.3 mmHg) (time per treatment interaction $p < 0.0001$). SBP measured by the participants decreased by 6.5 ± 8.3 mmHg in the Symbiosal® group (from 134.5 ± 4.3 to 128.0 ± 8.3 mmHg) and by 1.2 ± 6.8 mmHg in the control group (from 133.4 ± 4.1 ± 132.2 ± 8.6 mmHg) (time per treatment interaction $p = 0.04$).

The proportion of subjects whose SBP was under 130 mmHg at the end of the intervention was 77% in the Symbiosal® group vs 11% in the control group ($p < 0.0001$) in the measurements performed by investigators and 60% vs 37% ($p = 0.15$) in the measurements made by participants.

DBP values measured by the investigators significantly decreased in the Symbiosal® group (from 79.5 ± 6.8 to 74.7 ± 6.4 mmHg; −4.8 ± 6.6 mmHg) as compared to the control group (from 82.6 ± 7.8 to 85.3 ± 9.0 mmHg; 2.6 ± 6.5 mmHg; $p < 0.0008$ for time per treatment interaction). Similar results were obtained for DBP values measured by the participants ($p < 0.023$ for time per treatment interaction).

The Panel notes that 24-h urinary sodium excretion at baseline was higher in the control group compared to Symbiosal® group (146.8 ± 51.9 mmol/day vs 115.0 ± 51.6 mmol/day). Upon a request from EFSA, the applicant explained that the difference was not statistically significant ($p = 0.067$). The supplemented salt consumption measured by the weight of the saltcellar returned at the end of the study was not significantly different between groups (42.5 ± 21.8% of the saltcellar in the Symbiosal® group vs 47.0 ± 21.8% in the NaCl group, $p = 0.51$), which corresponded to a daily intake of 2.2 ± 1.1 g/day and 2.5 g/day of added salt, respectively. Urinary 24-h sodium excretion measured at the end of the intervention was not significantly different between groups (Symbiosal®: 127.3 ± 50.6 mmol/24 h, NaCl: 119.6 ± 42.8 mmol/24 h, $p = 0.61$). However, a time per treatment interaction was found when baseline and end of treatment values for 24-h urinary sodium excretion were considered ($p = 0.038$).

The applicant also provided calculations showing that, when adjusting for baseline values using analysis of covariance (ANCOVA), changes in 24-h urinary sodium excretion were not significantly different between groups ($p = 0.24$). Similar results were obtained after adjustment of urinary chloride for baseline values ($p = 0.72$). The applicant also performed ANCOVA to assess changes in BP during the study using either baseline 24-h urinary sodium excretion or changes in 24-h urinary sodium excretion during the study as covariates. Differences in SBP changes between groups remained statistically significant ($p < 0.0001$ for both calculations).

Mean body weight increased during the intervention in both groups by about 0.5 kg.

The Panel considers that this study with some methodological limitations (the process of enrolment was not well described, four subjects who did not meet the inclusion criteria were included in data analysis) shows an effect of Symbiosal® on SBP and DBP as compared to table salt when consumed in doses up to 3 g/day for 8 weeks in the context of a self-selected diet. The Panel notes that the mean difference in SBP changes between the Symbiosal® and the table salt groups was about 10 mmHg.

Allaert (2015) published the results of an uncontrolled intervention study in elderly subjects living in a rehabilitation centre who were asked to replace table salt with Symbiosal® in their usual diet. The Panel considers that no conclusions can be drawn from this uncontrolled study for the scientific evaluation of the claim.

Kato et al. (1994) measured arterial BP and serum ACE activity in seven healthy males (age 23–27 years) who consumed 5 g of Symbiosal® daily for 7 days. The Panel considers that no conclusions can be drawn from this uncontrolled study for the scientific substantiation of the claim.

The Panel notes that one human intervention study with methodological limitations from which conclusions could be drawn showed an effect of Symbiosal® on blood pressure as compared to table salt when consumed in doses up to 3 g/day for 8 weeks in the context of a self-selected diet. The Panel also notes that the results of the study have not been replicated by different research groups.

Animal efficacy studies

The applicant provided three publications reporting on animal efficacy studies (Kato et al., 1994; Je et al., 2006; Kim et al., 2010). The Panel also considered as pertinent to the claim two other publications on animal efficacy studies (Kim et al., 2005; Park et al., 2009), which were submitted with the previous application and were evaluated by the Panel in its scientific opinion (EFSA NDA Panel, 2015).

All the studies were reported to have been performed with chitosan-containing salts. The applicant claims that, even though it is not mentioned in the publications, the chitosan salt used in the studies was Symbiosal®, produced according to the patented manufacturing process.

In the publication by Je et al. (2006), the statistical analysis of the results related to blood pressure is not reported. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim. In the study by Kato et al. (1994), the comparator used (a high salt diet containing alginic acid) does not comply with the specifications given for the comparator for the claim (table salt). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

The publication by Kim et al. (2010) reports on two animal studies and one *in vitro* study. One animal study was carried out in normotensive Sprague–Dawley rats and did not find any effects of Symbiosal®) on BP compared with table salt. The second study was performed in spontaneously hypertensive rats (SHR) fed for 5 weeks (i) control diet (0% salt), (ii) control diet plus captopril, (iii) a diet with 3% Symbiosal®) or (iv) a diet with 3% commercial salt (six rats per group). After 5 weeks, the rats in Symbiosal®) group had a significantly lower SBP (two-way ANOVA, $p < 0.001$,) than rats on the diet with table salt.

In one animal study (Kim et al., 2005), SHRs were fed for 8 weeks a diet containing Symbiosal® (3%), a diet containing commercial salt (3%) or a control diet (seven rats per group). At the end of the study period, no significant differences in BP between the groups were reported.

In the animal study by Park et al. (2009), SHRs were administered for 2 months with either (i) Symbiosal®), (ii) NaCl plus potassium chloride (KCl), (iii) NaCl, (iv) chitosan or (v) untreated control diet (five animals per group). The concentration of sodium given was 44 mmol (1 g of sodium) per day. There were no statistically significant differences in blood pressure between the animals receiving the three salts at the end of the study.

The Panel notes that all three studies from which conclusions can be drawn evaluated the effect of Symbiosal® on blood pressure in SHRs, and that the effect was also assessed in normotensive rats in one study. One out of three studies performed in SHRs showed a decrease in blood pressure when Symbiosal® was consumed instead of table salt (Kim et al., 2010) while the two other studies (Kim et al., 2005; Park et al., 2009) found no effect of Symbiosal® on blood pressure. The results of the study in normotensive rats did not show an effect of Symbiosal® on blood pressure (Kim et al., 2010).

The Panel considers that these animal studies do not support the results of the human study.

Additionally, the applicant submitted five narrative reviews (Whitescarver et al., 1984; Venkatesan and Kim, 2010; Wijesekara and Kim, 2010; Cheung et al., 2015; McCallum et al., 2015) which did not provide any original data which could be used for the scientific substantiation of the claim.

Mechanism by which the food could exert the claimed effect

The applicant claims that chitosan 'acts as a chelator of the Cl^- and therefore decrease its hypertensive effect. It forms a complex with anion Cl^- which is stable enough to limit partially its digestive absorption. Decreased amount of Cl^- within the plasma serum prevents him to stimulate the angiotensin converting enzyme (ACE) receptors'.

Tanaka et al. (2005) (also published as Schmidlin et al. (2005)) tested the hypothesis that in stroke-prone SHRs, the Cl^- component of dietary NaCl dominantly determines its pressor effect (salt-sensitivity). Stroke-prone SHs received nothing (control), NaCl alone, KCl alone, NaCl combined with $KHCO_3$ and NaCl combined with KCl. Cl dominantly appeared to determine the pressor effect induced with dietary NaCl, both with NaCl alone or combined with either KCL or $KHCO_3$. The authors concluded that the Na component of dietary NaCl is not selectively sufficient to induce a pressor effect in SHRSPs and that in these rats the Cl component of dietary NaCl dominantly determines the expression of salt sensitivity. The Panel notes that Symbiosal® or chitosan were not used in this study.

Two *in vitro* studies in which ACE activity was measured in the presence of chitosan salt were submitted (Je et al., 2006; Kim et al., 2010). ACE inhibitory activity was measured by spectrophotometry. In the study by Je et al. (2006), three chitin salts with different degree of deacetylation were evaluated. All of them exhibited ACE inhibitory activity (the salt with medium deacylation had the highest effect). In the study by Kim et al. (2010) a 3% solution of chitosan-salt showed an ACE inhibitory effect of $40.01 \pm 0.14\%$, while NaCl gave an effect of about 3% (value estimated from the graph) and captopril, a chemical ACE inhibitor, had an effect of $57.94 \pm 1.33\%$. The differences were reported as statistically significant ($p < 0.001$).

In another *in vitro* study (Park et al., 2008) six chito-oligosaccharides with different molecular weight and degree of deacylation showed a renin inhibitory activity measured by fluorescence.

ACE activity was measured in plasma in two of the animal efficacy studies discussed previously. The results of the study by Kim et al. (2005) were reported as descriptive statistics only. The Panel considers that no conclusions can be drawn from this study with respect to the ACE inhibitory activity of Symbiosal® *in vivo* in animals. In the study by Park et al. (2009), ACE-I and ACE-II concentrations measured in serum using rat angiotensin I and II enzyme immunoassay were no statistically different between the groups receiving Symbiosal® and table salt.

The Panel notes that the studies provided do not establish that Symbiosal® binds Cl^- in the gut, or that, upon oral administration, Symbiosal® has an ACE inhibitory activity through this or other mechanisms.

The Panel considers that no evidence has been provided in support of a mechanism by which Symbiosal® could induce a decrease in BP upon oral consumption as compared to table salt *in vivo* in humans. In this context, the Panel notes the low biological plausibility for the effect reported in the human intervention study by Allaert (2017a,b), where consumption of Symbiosal® (3 g/day as added salt) induced a decrease in SBP of about 10 mmHg as compared to table salt.

Weighing of the evidence

In weighing the evidence, the Panel took into account that one human study with methodological limitations showed an effect of Symbiosal® on BP as compared to table salt when consumed in doses up to 3 g/day for 8 weeks in the context of a self-selected diet. The Panel notes that the mean difference in SBP changes between the Symbiosal® and the table salt groups was of about 10 mmHg. The Panel also took into account that no other human studies in which these results have been replicated by different research group were provided, that the animal studies did not support the results of the human study, that no evidence was provided in support of a mechanism by which Symbiosal® could induce a decrease in BP upon oral consumption as compared to table salt *in vivo* in humans, and in this context, the low biological plausibility of the effect observed in the human intervention study. The Panel concludes that a cause and effect relationship has not been established between the consumption of Symbiosal® and lowering of blood pressure.

4. Conclusions

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

- The food/constituent, Symbiosal®, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'lowers the rising of blood pressure when used as a replacement of traditional table salt. The rising of blood pressure is a risk factor for hypertension'. The target population proposed by the applicant is the 'subjects presenting prehypertension (SBP 130 to 139 mmHg and/or DBP 80 to 89 mmHg), and subjects presenting recently discovered mild to moderate hypertension'. Lowering of blood pressure is a beneficial physiological effect. Increased blood pressure is a risk factor for hypertension.
- A cause and effect relationship between Symbiosal® and lowering of blood pressure has not been established.

Steps taken by EFSA

1) Health claim application on pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0467_DE). Submitted by Han-Biotech GmbH, Industriestrasse 1, 77731 Willstätt, Germany.
2) This application was received by EFSA on 3/01/2018.
3) The scientific evaluation procedure started on 17/02/2017.
4) On 14/03/2018, 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 26/03/2017 and was restarted on 19/04/2018, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
5) On 19/04/2018, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 19/04/2018).
6) During its meeting on 27/06/2018, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to Symbiosal® and lowering of blood pressure and reduced risk of hypertension.

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Abbreviations

ACE	angiotensin-converting enzyme
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BMI	body mass index
BP	blood pressure
DBP	diastolic blood pressure
ITT	intention to treat
KCl	potassium chloride
NaCl	sodium chloride
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
PP	per protocol
SBP	systolic blood pressure
SD	standard deviation
SHR	spontaneously hypertensive rats