European Food Safety Authority

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SCIENTIFIC OPINION

Water-soluble tomato concentrate (WSTC I and II) and platelet aggregation

Scientific substantiation of a health claim related to water-soluble tomato concentrate (WSTC I and II) and platelet aggregation pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2009-00229)

Adopted on 15 May 2009

PANEL MEMBERS

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SUMMARY

Following an application from Provexis Natural Products Limited submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of United Kingdom, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to water-soluble tomato concentrate (WSTC I and II) and reduction of platelet aggregation.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and/or claim including a request for the protection of proprietary data.

The food constituent that is the subject of the health claim is a lycopene-free and fat-free water-soluble tomato concentrate (WSTC) developed in two variant forms named WSTC I (completely water-soluble syrup) and its low-sugar derivative, WSTC II, supplied in powder format. The WSTCs are standardised on the total quantity of 37 identified constituents which have been shown to inhibit platelet aggregation *in vitro* to different degrees. The Panel considers that the food constituents WSTC I and II are sufficiently characterized.

[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from Provexis Natural Products Limited on Water-soluble tomato concentrate (WSTC I and II) and platelet aggregation. *The EFSA Journal* (2009) 1101, 1-15

The claimed effect is "reduction in platelet aggregation". The target population is healthy adults between 35 and 70 years of age. The Panel considers that maintaining normal platelet aggregation is beneficial to human health.

The substantiation of the claimed effect is based on eight human studies (seven claimed as proprietary and conducted with WSTC) and seven (three claimed as proprietary) non human studies.

In the seven human intervention studies claimed as proprietary, the effects of WSTC on platelet aggregation *ex vivo* was investigated in carefully selected male and female subjects between 35 and 70 years of age. The Panel considers that both the selection of subjects and the method used to assess platelet aggregation were appropriate for such studies.

In a double-blinded randomized controlled trial (RCT), a significant reduction (compared to placebo) of 8-25% in platelet aggregation was observed 3 h after consuming tomato extract corresponding to 3 g and 9 g of WSTC I in 200 mL orange juice. In a single-blinded crossover RCT a significant reduction (compared to placebo) in platelet aggregation was observed between 1.5 and 3 h after consumption of the 9 g WSTC I in either 50 or 250 mL of orange juice, which persisted for 12 h. In a non controlled crossover study platelet aggregation was inhibited by 7-8 % at 12 h, but returned to baseline values at 18 and 24 h following consumption of a single dose of 3 g of WSTC I. In a double-blinded, crossover RCT, platelet aggregation was significantly reduced (compared to tomato-free control drink) after 14 and 28 days of daily consumption 3 g of WSTC I in 200 mL orange juice.

In a crossover RCT a significant reduction in platelet aggregation was observed 3 h after consuming a single dose of 250 mL (but not with a single dose of 1L) of a fruit juice drink containing 12 g of WSTC I/L. Repeating the test after subjects had consumed 1L of the WSTC drink daily for 5 days resulted in a similar outcome. In a pilot, non-controlled study platelet aggregation was significantly reduced (compared to baseline) 3 h after consuming a single dose of 3 g of WSTC I in 250 mL orange juice and after a single dose of 150 mg or 600 mg of WSTC II in 100 mL yoghurt drink, with no significant differences between the three preparations. In a double-blind, crossover RCT, platelet aggregation was reduced (compared to control and baseline) by a similar amount (13.5-17.2 %) 3 h after consuming 3 g WSTC I (syrup), 150 mg of WSTC II (powder) produced at ambient temperature or 150 mg of WSTC II (powder) produced at 65°C.

These human studies consistently show a reduction in platelet aggregation following consumption of WSTC under the conditions of use proposed by the applicant. Possible confounding factors likely to interfere with platelet aggregation were adequately addressed. Prothrombin and thrombin clotting times were not affected by supplementation with WSTC.

A double-blinded, parallel RCT showing a significant decrease (26.5%) in platelet aggregation following consumption of 250 mL filtered tomato juice (not WSTC) in diabetic subjects as compared to controls and seven non human studies identifying 37 compounds in aqueous tomato extracts with inhibitory activity against platelet aggregation *in vitro* were presented as supporting evidence.

The Panel notes a consistent effect of the supplementation with WSTC on platelet aggregation which is sustained for up to 28 days in subjects that are representative of the target population for which the claim is intended.

The Panel concludes that a cause and effect relationship has been established between the consumption of water-soluble tomato concentrate (i.e., WSTC I and II corresponding to the specifications provided by the applicant) and the reduction in platelet aggregation in humans.

The Panel could not have reached this conclusion without considering the studies claimed by the applicant as proprietary.

The following wording reflects the scientific evidence: “helps maintain normal platelet aggregation”.

In order to achieve the claimed effect, 3 g WSTC I or 150 mg WSTC II in up to 250 mL of either fruit juices, flavoured drinks or yogurt drinks (unless heavily pasteurised) should be consumed daily. The target population is adults between 35 and 70 years of age.

Key words: water soluble tomato concentrate, WSTC I, WSTC II, platelet aggregation, clotting time, adults

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BACKGROUND

Regulation (EC) No 1924/2006[2] harmonises the provisions that relate to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of that Regulation and are authorised in accordance with this Regulation and included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of that Regulation lays down provisions for addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of that Regulation, an application for inclusion in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, who will make the application and any supplementary information supplied by the applicant available to European Food Safety Authority (EFSA).

Steps taken by EFSA:

- The application was received on 30/01/2009.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and/or claim including a request for the protection of proprietary.
- The scientific evaluation procedure started on 30/01/2009.
- On 05/02/2009 and on 18/04/2009, the NDA Panel agreed on the List of Questions which requests the applicant to supplement additional particulars to accompany the application.
- The applicant submitted the responses to the NDA Panel List of Questions on 18/02/2009 and 05/05/2009.
- During the meeting on 15/05/2009, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to water-soluble tomato concentrate (WSTC I and II) and reduction of platelet aggregation.

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: water-soluble tomato concentrate and reduction of platelet aggregation.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of water-soluble tomato concentrate (WSTC I and II), a positive assessment of its safety, nor a decision on whether water-soluble tomato concentrate (WSTC I and II) is, or is

[2] European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

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.

ACKNOWLEDGEMENTS

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1. Information provided by the applicant

Applicant's name and address: Provexis Natural Products Limited, Thames Court, 1 Victoria Street, Windsor, Berkshire, SL4 1YB, United Kingdom.

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006.

1.1. Food/constituent as stated by the applicant

Water-soluble tomato concentrate (WSTC) in two variant forms named WSTC I and its low-sugar derivative, WSTC II. WSTC is derived from ripe tomatoes, *Lycopersicon esculentum*, and is intended for use in fruit juices, fruit flavoured drinks and yoghurt drinks.

1.2. Health relationship as claimed by the applicant

Water-soluble tomato concentrate (WSTC) contains naturally occurring anti-platelet compounds which have been shown to suppress blood platelet activity in healthy people after consumption. Consuming WSTC reduces platelet aggregation, thereby maintaining the blood in a fluid and low-coagulable state. This helps to maintain healthy blood flow, by preventing micro-aggregates forming within the circulation, and by preventing the adherence of platelets to blood vessel walls or fatty plaques. Platelet function is not completely suppressed, and an appropriate level is maintained so that platelets can aggregate upon vascular injury.

1.3. Wording of the health claim as proposed by the applicant

The claimed health benefit is a reduction in platelet aggregation, which contributes to overall vascular and cardiovascular health. The proposed health claim resulting from that health benefit represents a concept of the significance of platelet function in vascular health which is understood by the consumer.

Proposed health claim wording: "Helps to maintain a healthy blood flow and benefits circulation".

1.4. Specific conditions of use as proposed by the applicant

The target population for the intended health claim is healthy adults between the ages of 35 and 70.

3 g/d of WSTC #1 or 150mg/d WSTC #2 should be consumed in a beverage format, of volume between 50 mL and 500 mL.

WSTC is derived from tomatoes, and thus individuals with a known allergy to tomatoes or tomato products should avoid consuming any foods to which WSTC has been added. It is suggested that individuals using anticoagulants such as warfarin should not add WSTC to their daily diet.

Use of aspirin in conjunction with WSTC should similarly be avoided, although use as an analgesic for short-term relief of acute pain is not contraindicated.

2. Assessment

2.1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is a lycopene-free and fat-free water-soluble tomato concentrate (WSTC) developed in two variant forms named WSTC I (completely water-soluble syrup) and its low-sugar derivative, WSTC II, supplied in powder format. These two products are prepared from tomato (*Lycopersicum esculentum*) using patented processes and are intended for use as food ingredients in fruit juices, fruit flavoured drinks and yoghurt drinks. The manufacturing process is clearly described. Chemical specifications of the constituents are provided and batch to batch reproducibility has been demonstrated. The WSTCs are standardized on the total quantity of 37 "bioactive" constituents identified and quantified using RP-HPLC-MS which have been shown to inhibit platelet aggregation *in vitro* to different degrees. The presence of unspecified constituents amounts to 12 mg/g wet weight (44 mg/g solids) and 86 mg/g solids for WSTC I and II respectively. Based on the potentially bio-active compounds, 3 g WSTC I are considered as equivalent to 150 mg WSTC II, and correspond approximately to the water soluble content of 2.5 tomatoes.

A number of physico-chemical characteristics have been assessed during stability testing, including breakdown products, pH, browning index, microbial status, ascorbate content, free amino-acids, free uronic acids, free glucose and fructose, Amadori products, Maillard products. From these tests it is indicated that WSTC I and II formulations are stable for 12 and 6 months respectively. The WSTC 'bioactive' components have been shown to survive and to retain their 'bioactivity' *in vitro* at 4-6 ^{o}C over typical product shelf lives of 14-90 days when WSTC was included in fruit juices, fruit flavoured drinks and yoghurt drinks (but not in heavily pasteurised yoghurt drinks). The compatibility of WSTC in the presence of live yoghurt cultures has not been established.

Considering the manufacturing process and its results, the food (WSTC) has obtained the GRAS status (generally recognized as safe) in the USA and has been considered by the Food Standards Agency as not falling under the Novel Food Regulation[3].

The Panel considers that the food constituents, WSTC I and II, which are the subject of the claim are sufficiently characterized.

2.2. Relevance of the claimed effect to human health

The claimed effect is "reduction in platelet aggregation". The target population is healthy adults between 35 and 70 years of age.

The applicant has performed three well-described literature searches to provide a rationale for the health benefits of reducing platelet aggregation in humans. Platelet hyperactivity and hypercoagulability states are more commonly observed in subjects presenting cardiovascular (CV) risk factors (e.g., smokers, hypercholesterolaemic subjects, obese, diabetic) and have been shown to play a role in the development of atherosclerosis and its complications. The development of different anti-platelet therapies has been a target for the prevention and treatment of cardiovascular disease (Graham *et al*., 2007).

The applicant argues that healthy subjects at very low risk of CV disease (i.e, without risk factors) normally have non-activated circulating platelets, and that decreasing platelet aggregation in subjects with constitutive platelet activation would contribute to "normalise" or

[3] European Parliament and Council (2007). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 043, 14.02.1997, p. 1-6.

"restore" a "normal" platelet function, which may be relevant in the context of delaying atherosclerosis progression and cardiovascular complications.

The Panel considers that maintaining normal platelet aggregation is beneficial to human health.

2.3. Scientific substantiation of the claimed effect

The applicant performed a literature search through three databases (Cochrane, Medline, Embase) using different combinations of keywords related to platelets and platelet activity (including haemostasis, thrombosis, cardiovascular disease) and tomato (or tomato extract/product) for retrieving controlled intervention studies in humans. The search strategy is clearly defined. A total of eight published studies were identified through the search. After application of clear exclusion criteria, the substantiation of the claimed effect is based on three published randomized human studies (Lazarus *et al*., 2004; O'Kennedy *et al*., 2006a, 2006b) and five unpublished human studies (three randomized controlled trials and two non-controlled trials; O'Kennedy *et al*., 2003a, 2003b, 2005, 2006c, 2007). Five published (Dutta-Roy *et al*, 2001; Lazarus and Garg, 2003, 2004; O'Kennedy *et al*. 2006b, Yamamoto *et al*., 2003) and two unpublished non human studies (Song *et al*., 2008; Zhang *et al*., 2008) were also considered by the applicant as supportive. The Panel considers that all the studies identified above are pertinent to the substantiation of the health claim.

All of the following human intervention studies are claimed by the applicant as proprietary (O'Kennedy *et al*., 2003a, 2003b, 2005, 2006a, 2006b 2006c, 2007). A large set of exclusion criteria for the selection of participants was applied in all of the studies in an effort to minimise confounding factors affecting platelet aggregation. About 10% of the subjects screened were excluded for presenting low platelet function in response to 3 μmol/L adenosine diphosphate (ADP) agonist. The Panel considers these exclusion criteria to be appropriate.

In the human intervention studies (O'Kennedy *et al*., 2003a, 2003b, 2005, 2006a, 2006b 2006c, 2007), the effects of tomato extracts on platelet aggregation were tested *ex vivo* as the percent inhibition in platelet aggregation in response to different platelet agonists (i.e., ADP, collagen, thrombin receptor activating peptide agonist (TRAP) or arachidonic acid). Platelet aggregation was determined *ex vivo* using the light transmission aggregometry methodology (Michelson, 2009). The Panel considers that the method used in these studies is appropriate to measure platelet aggregation in clinical studies.

In a double-blinded RCT (three-way crossover design), O'Kennedy *et al*. (2006a) determined the acute effects of two doses of tomato extract corresponding to 3 g and 9 g of WSTC I in 200 mL orange juice taken on a single occasion versus placebo (same volume of a tomato-free flavoured drink) on platelet aggregation. Following power calculations based on the expected response to suboptimal 3 μmol/L ADP concentration, 90 subjects (40 female) 45-70 years were recruited and completed the study. A significant reduction in platelet aggregation was observed 3 h after supplementation with the 3 g and the 9 g doses of tomato extract as compared to the control drink. Significant differences between the 3 g and the 9 g doses were observed only at suboptimal ADP concentrations. Out of the 90 subjects, 87 responded at least to one agonist (only three subjects were non responders for both agonists), whereas around 40-50 % appeared to be non responders to the supplementation either for ADP or for collagen. Overall, average inhibition of platelet aggregation was in the range 8-25%.

O'Kennedy *et al*. (2006b) assessed platelet aggregation in response to ADP, collagen, thrombin and arachidonate in cannulated male and female individuals aged 40-65 years after the administration of either placebo or tomato extract corresponding to 9 g of WSTC I in either 50 or 250 mL of freshly squeezed orange juice (4 study arms). The study was a single-blinded

RCT with a cross-over design using 10-day washout periods and including five subjects in the two control arms and 3-9 subjects in the two intervention arms (owing to drop outs for various reasons). A significant reduction from baseline in platelet aggregation was observed between 1.5 and 3 h after consumption of the 9 g WSTC I on either 50 or 250 mL at suboptimal (3 µmol/L) ADP concentration as compared to placebo, but not at optimal ADP concentrations (7-8.5 µmol/L). The effect observed at sub-optimal ADP concentrations persisted for 12 h. The Panel notes that doses used in this study were three times higher than those proposed in the conditions of use.

The unpublished study by O'Kennedy *et al*. (2003a) was performed to establish the persistence of the effect in the reduction of platelet aggregation observed after consumption of a single dose of WSTC I (3 g) in healthy male and female volunteers aged 40-65 years in a non controlled crossover study including 15 subjects in each of the three intervention arms (12, 18 and 24 h after the supplementation). Platelet aggregation was inhibited by 7-8 % at 12 h, but was not different from baseline at 18 and 24 h after consumption of WSTC I.

The unpublished study by O'Kennedy *et al*. (2003b) was designed to test the chronic effect (one month) of continuous supplementation with tomato extract corresponding to 3 g of WSTC I in 200 mL orange juice on platelet aggregation in a randomised, double-blinded, placebo controlled crossover trial (22 subjects per arm). Significant inhibition of platelet aggregation in response to 3 µmol/L (suboptimal) and 7.5 µmol/L (optimal) ADP concentrations compared to baseline values and to a control group given tomato-free supplement prior to the intervention was observed after two and four weeks of WSTC I consumption (11-33 % for suboptimal ADP concentration; 5.5-11.7 % for optimal ADP concentration). A carryover effect (7.7 % platelet inhibition) was observed two weeks after the end of WSTC I supplementation suggesting that the effects on platelet function were not simply direct anti-platelet effects. No effect was observed on the other variables studied (e.g., serum triglycerides, plasma homocysteine, CRP).

The unpublished study by O'Kennedy *et al*. (2005) was designed to examine the effects of consuming WSTC at levels greater than recommended in the proposed conditions of use. The acute effects of consuming a single dose of 250 mL and 1 L of a fruit juice drink containing 12 g WSTC I / L (3 g and 12 g of WSTC I, respectively) on platelet aggregation were compared. It was a randomized study following a crossover design in healthy volunteers 45-70 years old having shown a high sensitivity (>25 % inhibition to agonists) in previous studies. Three hours after consuming the 250 mL dose, a significant reduction in platelet aggregation was observed (12-14 % for ADP, 15-20 % for TRAP), whereas no significant effect was observed for the 1 L dose. Repeating the test after subjects had consumed 1 L of the fruit juice drink containing 12g WSTC I / L for 5 days resulted in a similar outcome. The study confirmed the effect of WSTC on platelet aggregation when consumed in a volume of 250 mL and indicated that there was no significant effect of WSTC when consumed in volume of 1 L. It was suggested that the lack of effect of WSTC when consumed in large volumes of liquid might have been owing to altered absorption profile of the active components.

The objective of the unpublished pilot, non-controlled study by O'Kennedy *et al*. (2006c) was to compare the effects of WSTC in different matrices (fruit juice and yoghurt) on platelet aggregation in subjects previously defined as responders to WSTC (healthy males and females 45-70 years old, nine subjects randomised to each arm). One dose of 3 g of WSTC I in 250 mL orange juice and two doses (150 mg and 600 mg) of WSTC II in 100 mL yoghurt drink were used for testing. Inhibition of platelet aggregation (suboptimal 3 µmol/L ADP concentration) 3 h after supplementation was statistically significant for all drinks tested as compared to baseline (18.4 % for fruit juice, 27.5 % for WSTC II 600 mg in yoghurt drink and 13.7 % for WSTC II 150 mg in yoghurt drink). For the optimal (7.5 µmol/L) ADP concentration, the respective inhibitions were 10.5%, 6.5 % and 4.9 % (all statistically significant). For collagen

(3 mg/L), the inhibition was significant only for the fruit juice and the WSTC II 600 mg. The inhibition of platelet aggregation was not statistically significant for collagen 5 mg/L, nor for the TRAP agonist for any tested food. No significant differences between the three drinks were observed for any of the tests used in the study. The absence of dose-response effect following consumption of different doses of WSTC II in yoghurt (150 mg and 600 mg) is tentatively explained by matrix effects on the bioavailability of WSTC II. The Panel considers that this study supports an equivalent effect of 3 g WSTC I in 250 mL orange juice and of 150 mg WSTC II in 100 mL yoghurt drink on platelet aggregation.

The unpublished study by O'Kennedy *et al*. (2007) addressed the acute effects of different forms of the tomato extract on platelet aggregation. The study was a double-blind placebo-controlled, randomized crossover design with three interventions (corresponding to 3 g WSTC I (syrup), WSTC II 150 mg (powder) produced at ambient temperature, and WSTC II 150 mg (powder) produced at 65°C) and one control, with 45 healthy males and females 35-70 years old on each arm. The results showed that the three WSTC formulations reduced platelet aggregation (ADP agonist) as compared to the corresponding control and baseline values, ranging from 13.5 to 17.2 %, with no significant differences between the three formulations. The responses to collagen 2 mg/L were also significant for all three formulations, whereas the response to collagen 5 mg/L was significant only for WSTC I. Thromboxane A2 generation (measured by the concentration of the stable metabolite thromboxane B2) was significantly reduced by the three formulations for ADP and both collagen concentrations. No changes were observed in plasma soluble P-selectin, a marker of platelet activation.

Taken together the human studies described above consistently show a reduction in platelet aggregation following consumption of WSTC at suboptimal ADP concentrations under the conditions of use proposed by the applicant. Possible confounding factors likely to interfere with platelet aggregation have been addressed and the within subject variability accounted for in the studies.

Prothrombin and thrombin clotting times were not affected by the supplementations in any of the studies above, suggesting a lack of effect in the clotting cascade and thus a lack of increase in bleeding risk. Consumption of WSTC at doses up to four times the intake proposed in the conditions of use did not lead to increased inhibition in platelet aggregation.

A study published as a letter to the editor was presented in support of the claimed effect. Lazarus *et al*. (2004) examined the effects of consuming filtered tomato juice (not WSTC) on inhibition of platelet aggregation in a double-blinded randomized controlled trial (RCT) in two parallel arms including male and female subjects 43 to 82 years of age with either diabetes type 2 (n =18) or impaired glucose tolerance (n = 2) and without history of thromboembolic events. Subjects were randomised to consume either 250 mL of filtered tomato juice or the same volume of water with tomato flavour (controls) for 21 days. Tomato intake was controlled using a 3-day weighed food record. The supplementation with tomato juice resulted in a significant 26.5% decrease in *ex vivo* platelet aggregation induced by collagen as compared to a non significant 2.4 % decrease in controls. No changes were observed during the study or between groups in glycaemic control assessed by HbA1c.

Three out of the seven non human studies provided are claimed as proprietary by the applicant and have been conducted with WSTC (O'Kennedy *et al*., 2006b; Song *et al*., 2008; Zhang *et al*., 2008). These three studies aimed to identify the spectrum of components in WSTC with inhibitory effects on platelet aggregation *in vitro* by sub-fractionating aqueous tomato extracts, and to establish IC50 values for inhibition of ADP-, collagen-, arachidonic acid- and thrombin-induced platelet aggregation for the 37 identified "active" components (see section 2.1). The pre-treatment of platelets with tomato extracts (and different sub-fractions) decreased the expression of the active conformation of glycoprotein IIb-IIIa, thus decreasing the ability of

fibrinogen to bind to platelets. Expression of P-selectin, involved in targeting activated platelets to vessel walls, was also reduced in treated samples.

Three additional non-proprietary *in vitro* studies (Dutta-Roy *et al*., 2001; Lazarus and Garg 2003 and 2004) and one *in vivo* animal study (Yamamoto *et al*., 2003) were presented in support of the claim.

The study by Dutta-Roy *et al*. (2001) demonstrated *in vitro* inhibition of platelet aggregation of delipidated tomato extracts in response to various agonists (i.e, ADP, collagen, thrombin). The inhibition was attributed in part to the adenosine content, as it was partially abolished by adenosine deaminase treatment. Lazarus and Garg (2003) confirmed the *in vitro* inhibition of platelet aggregation of delipidated tomato extract and ruled out the alteration of platelet cAMP metabolism as a possible mechanism. The same authors (Lazarus and Garg, 2004) showed an *in vitro* synergistic interaction between delipidated tomato extracts and omega-3 fatty acids on platelet aggregation. Finally, Yamamoto *et al*. (2003) showed an inhibitory effect on platelet aggregation of centrifuged and filtrated tomato juice, with a decrease in efficacy with ripening. They also observed an inhibitory effect of tomato extract on platelet aggregation *in vivo* in a mouse model using laser-induced thrombosis.

The Panel notes a consistent effect of the supplementation with WSTC on platelet aggregation which is sustained up to 28 days in subjects that are representative of the target population for which the claim is intended. The biological plausibility of this effect is supported by the presence of 37 identified compounds in aqueous tomato extracts showing different degrees of inhibition of platelet aggregation *in vitro* and by the effects of tomato extract on markers of platelet function in the animal study.

The Panel concludes that a cause and effect relationship has been established between the consumption of water-soluble tomato concentrate (i.e., WSTC I and II corresponding to the specifications provided by the applicant) and the reduction in platelet aggregation in humans.

The Panel could not have reached this conclusion without considering the studies claimed by the applicant as proprietary (O'Kennedy *et al*., 2003a, 2003b, 2005, 2006a, 2006b 2006c, 2007; Song *et al*. 2008; Zhang *et al*., 2008).

2.4. Panel's comments on the proposed wording

The Panel considers that the following wording reflects the scientific evidence:

"Helps maintain normal platelet aggregation".

The Panel considers that the wording proposed by the applicant "helps to maintain a healthy blood flow and benefits circulation" does not reflect the scientific evidence because only measures of platelet aggregation have been used in the studies presented whereas "blood flow", and particularly "circulation", depend on many other factors that have not been addressed in the studies provided.

2.5. Conditions and restrictions of use

The Panel considers that, in order to achieve the claimed effect, 3 g WSTC I or 150 mg WSTC II in up to 250 mL of either fruit juices, flavoured drinks or yogurt drinks (unless heavily pasteurised) should be consumed daily. The compatibility of WSTC with the presence of live yoghurt cultures has not been established. The target population is adults between 35 and 70 years of age.

Although the applicant proposed that subjects with known allergy to tomato and/or tomato products should be advised to avoid consumption of WSTC-containing food products, the

Panel notes that tomato and products thereof are not included in the list of ingredients/substances or their derivatives for which labelling is mandatory in the European Union (Annex IIIa of Directive 2003/89/EC [4]).

The applicant also proposed that individuals using anticoagulants such as warfarin and individuals using aspirin on a regular basis should be advised not to add WSTC-containing food products in their daily diet. However, subjects taking either warfarin or aspirin on a regular basis are generally not advised to avoid consumption of tomato and/or tomato products in medical practice and no convincing rationale has been provided for such restrictions of use in the application.

CONCLUSIONS AND RECOMMENDATIONS

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

- The food constituents, WSTC I and II, which are the subject of the claim are sufficiently characterized.
- The claimed effect is "reduction in platelet aggregation". The target population is healthy adults between 35 and 70 years of age. Maintaining normal platelet aggregation is beneficial to human health.
- A cause and effect relationship has been established between the consumption of water-soluble tomato concentrate (i.e., WSTC I and II corresponding to the specifications provided by the applicant) and the reduction in platelet aggregation in humans.
- The following wordings reflects the scientific evidence: "helps maintain normal platelet aggregation"
- The Panel considers that, in order to achieve the claimed effect, 3 g WSTC I or 150 mg WSTC II in up to 250 mL of either fruit juices, flavoured drinks or yogurt drinks (unless heavily pasteurised) should be consumed daily. The compatibility of WSTC in the presence of live yoghurt cultures has not been established. The target population is adults between 35 and 70 years of age.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on water-soluble tomato concentrate (WSTC I and II) and reduction of platelet aggregation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0235-UK). February 2009. Submitted by Provexis Natural Products Limited.

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GLOSSARY / ABBREVIATIONS

ADP	Adenosin diphosphate
CRP	C-reactive protein

IC50	Half maximal inhibitory concentration
RP-HPLC-MS	Reverse phase-high-performance liquid chromatography-mass spectrometry
TRAP	Thrombin receptor activating peptide agonist
WSTC	Water soluble tomato concentrate