

Safety Assessment of *Ecklonia cava* phlorotannins (SeaPolynol™)

Regulation (EC) No. 258/97

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Novel Food Category: 1.2(d)

Introduction

An application for the authorisation of *Ecklonia cava* phlorotannins (SeaPolynol™) as a novel food ingredient was submitted to the Food Safety Authority of Ireland (FSAI) by Botamedi Inc. of Korea in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on May 14th 2015.

The novel ingredient is an alcohol extract of the seaweed *Ecklonia cava* and is intended as an ingredient in food supplements for the EU market. The main constituent of SeaPolynol™ is a class of polyphenols (antioxidants) called phlorotannins, secondary metabolites found exclusively in certain brown algae. They are structurally distinct from plant-based polyphenols (such as resveratrol or quercetin) in that they are made up of phloroglucinol polymers (1,3,5-trihydroxybenzene).

Several types of food supplements containing SeaPolynol™ are available on the market in Asia and the United States (US), though it seems that those products are not necessarily the same formulation as the product proposed in this application. SeaPolynol™ does not have a history of food use in the EU and therefore requires pre-market authorisation under the novel food Regulation. The maximum recommended daily intake for SeaPolynol™ initially proposed by the applicant was 450 mg/day which equated to 428 mg/day of phlorotannins based on 95% purity. Following consultation with the applicant, this was lowered to a recommended intake of 360 mg/day of SeaPolynol™ (342 mg/day of phlorotannins), taking into account that the maximum dose used in the four clinical trials was 400 mg/day (360 mg/d phlorotannins). Though this recommended intake is equivalent to that for similar products on some Asian markets, it is considerably higher than that recommended in the USA (47 mg phlorotannins/day), though this was subsequently explained by the applicant. SeaPolynol™ is intended for general use as a food supplement by healthy individuals over the age of 12 years, with no other restrictions proposed.

SeaPolynol™ falls within *Article 1.2(d)* of the novel food Regulation (EC) No 258/97 “*foods and food ingredients consisting of or isolated from microorganisms fungi or algae*”. In order to assess wholesomeness, the application dossier was prepared pursuant to Class 2 of Commission Recommendation 97/618/EC; “*Complex novel food from a non-GM source*”, and sub-class (2); “*the source of the novel food has no history of food use in the Community*”.

I. Specification of the novel food

SeaPolynol™ is produced as a brown powder with the predominant constituent being phlorotannins, a class of polyphenols (antioxidants) occurring naturally as secondary metabolites in brown algae. Since phlorotannins are a complex mixture with no absolute standard available for efficient quantification purposes, the applicant uses a polyphenol-reactive coloration technique (Folin-Ciocalteu method) with a commercially available surrogate chemical (phloroglucinol, a simple polyphenol) to assess the phlorotannin content of SeaPolynol™ for the purposes of Quality Control. Using the Folin-Ciocalteu method, SeaPolynol™ was shown to contain at least 95% phlorotannins, which is the minimum phlorotannin content specified by the applicant. Analysis of six batches of SeaPolynol™ using this method resulted in an average phlorotannin content of 97.7%. The remaining content of SeaPolynol™ is made up of moisture, ash, protein and fat in decreasing levels.

Approximately 57% of the phlorotannins in SeaPolynol™ have been identified, with HPLC analysis identifying Dieckol, 8,8'Bieckol and 7-Phloroeckol as the predominant compounds present. The applicant proposes that NMR analysis shows that all detected compounds in SeaPolynol™ are polymers of phloroglucinol (i.e. phlorotannins) commonly available in brown algae. However, it appears many of these polymers have yet to be fully characterised. The applicant contends that the production process for SeaPolynol™ is similar to production processes traditionally used for seaweed products, and is therefore not novel. Compositional and quality specifications for the novel ingredient are defined by the applicant and supported by analytical results from six lots of SeaPolynol™. Each lot of SeaPolynol™ undergoes quality control checks for phlorotannin profiles, antioxidant activity, ash, moisture, heavy metals and microbial purity. Other contaminants routinely monitored include inorganic arsenic, pesticides, dioxins, PCBs and aflatoxins. There have been no reports to date of allergic reactions associated with the consumption of supplements containing SeaPolynol™ which have been sold on Asian (South Korea and Japan) markets since 2004 and in the US since 2006.

II. Effect of the production process applied to the novel food

SeaPolynol™ is manufactured in a process similar to that used in traditional seaweed processing and in accordance with GMP standards and HACCP principles. The initial ISO 22000:2005 certificate provided by the applicant expired in January 2015, but this has been replaced by a new certificate which is valid until January 2018. Production begins with fresh, semi dried *Ecklonia cava* seaweed which is dried, crushed and subjected to extraction, filtration and purification steps. The applicant notes that *Ecklonia cava* is abundant in southern Japan and South Korea, and the location of the seaweed source has been confirmed as Jeju Island in South Korea. The alcohol used in the extraction process has been clarified by the applicant to be food grade ethanol. Each seaweed lot is visually inspected upon arrival for processing and undergoes quality analysis for several specified parameters.

III. History of the source organism

Ecklonia cava is a brown algae abundant in the seas off central and southern Japan as well as South Korea. Generally, brown algae can be used in food directly or as a source of alginates which have numerous industrial, pharmaceutical, agricultural and food applications. Many Asian and other countries have a significant history of consumption of brown seaweeds which naturally contain low levels of phlorotannins.

The addition of *Ecklonia cava* to general foodstuffs in various Asian countries is also discussed by the applicant, along with its similarity to other closely related brown algae, including *Eisinia bicyclis* which has a history of human consumption in the EU prior to 1997.

IX. Anticipated intake/extent of use of the novel food

The applicant intends to market SeaPolynol™ in food supplements within the EU, with a recommended daily intake of 360 mg/day (reduced from 450 mg/day following consultation with the applicant). Though the level of phlorotannins present in 360 mg of SeaPolynol™ is not specified in the application, it can be estimated to represent an intake of approximately 342 mg/day of phlorotannins based on SeaPolynol™ being comprised of 95% phlorotannins. While this is similar to recommended dose levels in Korea, it is significantly higher than the intake of 47 mg/day recommended in the USA. However, the applicant has clarified that the US product was a crude phlorotannin extract (13% phlorotannin) which represented the first such seaweed extract marketed in a non-Asian country. The 47 mg/day recommended dose was proposed by the applicant based on a less complete toxicological dataset than was available in subsequent years.

SeaPolynol™ will be targeted at healthy adults and children over 12 years of age, with no other restrictions such as time limits for consumption or advice labels for any potential vulnerable groups proposed. Additional sales data was provided to demonstrate consumption patterns in Asian markets, and to a limited extent in the US market. The applicant contends that SeaPolynol™ is a new supplement for the EU market and they have provided additional data on predicted overall consumption levels for the first five years on the EU market.

X. Information from previous human exposure to the novel food or its source

Ecklonia cava extract has not been consumed in the EU and therefore requires authorisation under the novel food Regulation. However, food supplements containing SeaPolynol™ have been on the market in Japan and Korea since 2004 and in the US since 2006 where adverse event reporting structures are in place. In addition, many Asian and other countries have a significant history of consumption of brown seaweeds which naturally contain low levels of phlorotannins. The applicant has provided copies of advertising material which demonstrates that *Ecklonia cava* is sold as an ingredient in food supplements available in South Korea and the USA. The applicant also provides a copy of the Korean FDA's authorisation to sell

SeaPolynol™ without restriction, and the US FDA letter in response to the notification of a dietary supplement (Seanol-F) which also contains *Ecklonia cava* extract. The 2008 US FDA letter is an acknowledgement of the company's notification rather than a safety statement *per se*, and informs the notifier that the FDA has no objection to the marketing of Seanol-F at the levels proposed (47 mg phlorotannins/day) to adults and children over the age of twelve years. The applicant has clarified that this product was a crude extract (13% phlorotannins) and the recommended intake was somewhat cautious as it was the first time of marketing this product outside of Asia. In 2008, the available safety data on phlorotannin consumption was relatively limited. However, the Korean authorities reviewed more recent safety data in 2012 and approved a recommended intake level similar to that proposed for the EU market. The US authorities had some concerns in 2008 about the marketing of Seanol-F to children less than 12 years of age and the applicant has agreed to restrict the marketing of SeaPolynol™ in the EU to healthy adults and children over 12 years of age, while providing the following information and advisory statements:

- a) The names of the categories of nutrients or substances that characterise the product or an indication of the nature of those nutrients or substances;
- b) The portion of the product recommended for daily consumption;
- c) A warning not to exceed the stated recommended dose;
- d) A statement to the effect that food supplements should not be used as a substitute for a varied diet;
- e) A statement to the effect that the products should be stored out of the reach of young children.

XI. Nutritional information on the novel food

The novel food ingredient is not intended to replace any foods in the EU diet, but may replace other algal sources of vitamins, minerals (e.g. iodine) and components specific to algae (e.g. fucoxanthin, fucosterol and phlorotannin). Compositional analysis shows that 100 g of SeaPolynol™ has an energy content of 378 ± 2 kcal and is composed of 90.2% carbohydrate/phlorotannin, 2.5% crude protein, 0.8% crude fat, 3.2% ash and 3.4% moisture. The phlorotannin concentration is calculated from the carbohydrate content with presence confirmed by NMR and HPLC. Minerals present in relatively higher quantities include sodium ($4,400 \pm 800$ mg/kg), calcium ($4,800 \pm 400$ mg/kg), magnesium ($1,300 \pm 100$ mg/kg), potassium (700 ± 200 mg/kg) and iodine (220 ± 40 mg/kg). The main phlorotannins identified by HPLC are Dieckol (21% of total), 8,8,-Bieckol and 7-Phloroieckol (14%), 2-O-(2,4,5-trihydroxyphenyl)-6,6'-bieckol (6.5%) and 6,6'-Bieckol (6.15%), with 5 other phlorotannins listed and 43.3% listed as 'unidentified'. This suggests that a significant proportion of the phlorotannins present in *Ecklonia cava* are as yet uncharacterised. In addition, there is limited information available on the absorption, metabolism or fate of SeaPolynol™ or the constituent phlorotannins, though a recent publication in early 2016 provides some information on the metabolism and bioavailability of phlorotannins from the brown seaweed *Ascophyllum nodosum*. The applicant refers to potential health claims

within various jurisdictions but these are not considered relevant for this safety assessment.

XII. Microbiological information on the novel food

Microbiological analysis of SeaPolynol™ demonstrates the absence of coliforms (including *E. coli*), *Staphylococcus aureus*, *Salmonella*, moulds and yeasts.

XIII. Toxicological information on the novel food

(i) Controlled contaminants

The applicant initially indicated control specifications of <4 mg/kg for lead, mercury and cadmium in SeaPolynol™ despite much lower levels being detected during batch analysis. However, following consultation, the applicant reduced the specifications for lead, mercury and cadmium to 3, 0.1 and 3 ppm respectively, in order to comply with maximum levels (MLs) of these metals permitted in food supplements in the EU (Commission Regulation (EC) 1881/2006). Though the level of inorganic arsenic in SeaPolynol™ is negligible, total arsenic averaged at 20.71 mg/kg (maximum of 23.40 mg/kg). This means that supplementation with SeaPolynol™ at 360 mg/day, assuming a total arsenic content of 23.40 mg/kg, would contribute a further 8.4 µg to the daily dietary intake. This represents an additional 13.2% exposure to total dietary arsenic of < 64 µg per day estimated in the 2006 UK Total Diet Study.

The Tolerable Upper Intake Level of iodine by adults was set at 600 µg/day by the Scientific Committee on Food in 2002. Considering that the maximum level of iodine detected in SeaPolynol™ batches was 281.6 mg/kg, a daily dose of 360 mg SeaPolynol™ represents a dose of 101.38 µg/day of iodine; giving a margin of exposure of 5.9. Following consultation with the applicant, the upper limit specification for iodine was lowered to 400 mg/kg, which means that a daily dose of 360 mg SeaPolynol™/day would correspond to a maximum intake of 144 µg iodine/day with a margin of exposure of 4.2.

The margins of exposure to minerals including cobalt, nickel, selenium, molybdenum, zinc, chromium and iron were acceptable in terms of either the established Tolerable Daily Intake or the Tolerable Upper Intake Level.

Analysis of six product batches for the presence of pesticides, seven PCB congeners, aflatoxins B1, B2, G1 and G2, or *Salmonella* did not highlight any concerns. Dioxins were detected in only one batch at 0.382pg-TEQ/g. Based on the Tolerable Weekly Intake (TWI) of 14 pg WHO-TEQ/kg body weight for dioxins and dioxin-like PCBs, and a dose level of 360 mg/day SeaPolynol™, the contribution to the TWI was approximately 0.01% which is negligible. Screening for 245 pesticide residues resulted in the detection of Azinphos-methyl and Phenthoate at levels that did not raise any safety concerns. However, the applicant has subsequently decided to change the harvest location in order to ensure the absence of pesticide residues.

(ii) Acute toxicity

The acute oral LD₅₀ of SeaPolynol™ (99.5% phlorotannin) was reported to be >2,000 mg/kg bw/day in 6 week old Sprague-Dawley rats and > 1,000 mg/kg bw/day in dogs. Aside from stool effects in rats and dogs as well as vomiting episodes in dogs, no mortalities, effects on body weight/body weight gain or other clinical abnormalities were recorded.

(iii) Sub-chronic and repeated dose toxicity

A dose-range finding study of SeaPolynol™ (93% phlorotannin) in Sprague-Dawley rats (0, 500, 1,000 or 2,000 mg/kg bw/day) did not result in any notable adverse effects. However, a high dose of 1,500 mg/kg bw/day was selected by the applicant for the 13-week sub-chronic toxicity study because of inconsistent effects on liver weight, liver pathology and associated blood chemistry parameters at the high dose of 2,000 mg/kg bw/day. There were no mortalities or treatment-related effects on food consumption or ophthalmology during the 13-week sub-chronic toxicity study. Dose-related decreases in organ weight were statistically insignificant and probably reflected the decrease in body weight as they were not corroborated by changes in relative organ weight. Observed changes in haematological, urinalysis, clinical chemistry, gross necropsy and histopathological parameters were unremarkable and were within historical controls as confirmed by the applicant. Compound coloured stools were recorded in the acute and sub-chronic toxicity studies in rats and dogs in addition to sporadic cases of gastrointestinal upset that were also recorded in clinical trials in humans. The applicant explained this was due to excretion of the test substance and was thus not considered to be of toxicological significance. Based on treatment-related decreases in body-weights observed in both sexes at 1,500 mg/kg bw/day, the NOAEL was determined to be 750 mg/kg bw/day.

(iv) Genotoxicity and mutagenicity

The genotoxic potential of SeaPolynol™ was investigated in three tests carried out to GLP and in accordance with Korean FDA test guidelines. The potential for a mutagenic effect was not identified in a bacterial reverse mutation test at SeaPolynol™ concentrations up to 5,000 µg/plate, with or without S9 fraction. Clastogenicity/aneugenicity were not evident in an *in-vivo* mouse micronucleus test in which 8-week old male mice were administered doses of 500, 1,000 and 2,000 mg/kg body weight by oral gavage. SeaPolynol™ was not deemed to influence chromosome aberrations in an assay system using Chinese hamster lung cells with or without S9 fraction.

(v) Clinical trials

The details of three published and one unpublished human clinical studies of SeaPolynol™ were provided by the applicant. All studies were carried out in Korea in accordance with Good Clinical Practice guidelines and no treatment-related adverse events or abnormal laboratory findings were reported.

(vi) Allergenicity

The applicant refers to evidence from scientific databases, traditional use, commercial consumption and clinical trials on the use of *Ecklonia cava* or SeaPolynol™ that indicates there is no known evidence of allergenicity associated with the consumption of *Ecklonia cava* or SeaPolynol™ to date. Furthermore, the applicant states that the US and Asian distributors of SeaPolynol™ have adverse event reporting procedures in place and to date have not had any reports of allergic reactions to this product.

Conclusions

The consumption of brown seaweed, including *Ecklonia cava* in a variety of foods is common in certain Asian countries and is not associated with any reports of negative health consequences. SeaPolynol™ is a food supplement consisting of a concentrated extract of *Ecklonia cava* (primarily phlorotannins) and is intended for the general population of healthy adults and children older than 12 years of age in the EU. The maximum recommended intake of SeaPolynol™ for the EU market is 360 mg/day (6 mg/kg bw/day for a 60 kg individual) which, at a purity of 95% represents a recommended intake of 342 mg/day of phlorotannins. While this recommended intake is similar to that for the Korean market, it represents a significant increase on the 47 mg/day of phlorotannins recommended for the US market. However, the recommended use level for the US market proposed by the applicant in 2008 was cautious for a few reasons; (1) The product was a crude extract (13% phlorotannin), (2) It represented the first time of marketing such a product in a non-Asian country and (3) The level proposed by the applicant was based on an incomplete toxicological dataset. A subsequent safety review in 2012 carried out by the Korean authorities resulted in the authorisation of higher recommended daily dose levels, similar to that proposed for the EU market.

Adverse effects have not been associated with the consumption of the novel ingredient in its various formulations or recommended intakes on Asian or US markets. Some additional details are provided on the consumption patterns of SeaPolynol™ in Asian and US markets and the applicant predicts general intakes in the EU for the first five years of marketing.

Children under the age of 12 are excluded from the target population in the US on the basis of concerns expressed by the US authorities at the time, and the availability of limited safety data. The applicant has agreed to market SeaPolynol™ only to healthy adults and children over the age of 12 years of age, while providing some additional information and advice through product labels. It should be considered whether further marketing restrictions should apply for other vulnerable groups such as people with certain illnesses (e.g. thyroid problems) and pregnant or breastfeeding women who are otherwise “healthy”, in order to be consistent with the cautious approach adopted in Korea.

A significant proportion of the phlorotannins present in *Ecklonia cava* are not yet fully characterised while data on the absorption, distribution, metabolism and excretion (ADME) of phlorotannins in the human body are limited.

Recommendation

The FSAI has not identified any direct health concerns associated with the consumption of SeaPolynol™ supplements at the dosage recommended by the applicant. In addition, the available information suggests that varying levels and formulations of dietary supplementation are well tolerated by US and Asian consumers, with no safety concerns identified.

However, the FSAI is of the opinion that an additional assessment is required in accordance with *Article 6.3* of the novel food Regulation to address a number of issues:

1. The consumption of seaweed and seaweed products in the EU is not as common as in Asian countries, and SeaPolynol™ supplements would be the first high dose phlorotannin supplement to be marketed outside of Asia,
2. A considerable proportion of phlorotannins in *Ecklonia cava* (and thus SeaPolynol™) have yet to be fully characterised,
3. The fate of phlorotannins (ADME) in the human body is not fully understood,
4. There is a need to consider whether other vulnerable groups in the EU such as pregnant or breastfeeding women and people with particular illnesses (e.g. thyroid problems) should consume this product,
5. A considerable amount of new data and clarifications have been provided by the applicant since the application dossier was initially assessed by FSAI, and these need to be fully considered alongside the original data to ensure a complete assessment.