ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

DRAFT OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR SPOROPOLLENIN SHELLS FROM CLUB MOSS

Applicant:	**Sporomex (UK) Ltd.**
Responsible Person:	**Grahame Mackenzie**
EC Classification:	**2.2**

Introduction

1. In January 2013, the Food Standards Agency accepted an application from Sporomex (UK) Ltd for sporopollenin shells from club moss (*Lycopodium clavatum*) as a novel ingredient. A copy of the application was placed on the Agency's website for public consultation.

2. All pollens and spores possess an outer shell called an exine, which protects the genetic material and nutrients. The exine or shell is made from a unique polymer, known as sporopollenin, which is composed of carbon, hydrogen and oxygen.

3. Sporopollenin shells (SS) are produced by emptying spores of their genetic, lipid and protein material to leave an empty sporopollenin shell. The intention is that the product will be filled with functional ingredients such as fish oils or vitamins (particularly vitamin D) or polyphenols. SS will therefore function as a system to deliver functional ingredients into the body. The novel ingredient plus its contents make a powder which could be incorporated into food or drink by the consumer or manufacturer.

4. The applicant states that this novel delivery system is intended so that functional ingredients are delivered more effectively into the body.

5. SS have been classified as a complex novel food from non-GM source. The source of the novel food has no history of food use in the EU (class 2.2) according to the scheme in Commission Recommendation 97/618/EC.

I. Specifications

6. The applicant's original dossier contained limited information relating to specifications of the novel ingredient, so the Committee requested further information that would allow formal specifications to be established, including

details of the molecular structure of sporopollenin, in addition to its physical characteristics.

7. The applicant provided additional information on a number of parameters as a result of the analyses of three separate samples of SS, including loss on drying, ash, proteins, lipids, carbohydrate, pesticides analyses, and elemental analysis including heavy metals. The applicant also carried out a molecular structural characterisation of sporopollenin and provided information on the relative amounts of carbon, hydrogen and oxygen, protein, carboxylic acid, hydroxyl groups, ester and ether groups, aldehydes and ketones, phenols and aromatic and aliphatic carbons. A formal specification of the product can be found at Appendix 1.

   ***Discussion**: The Committee reviewed the additional information submitted by the applicant and was content that this provided sufficient details to carry out a risk assessment. No further questions were raised on this section of the dossier.*

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

8. SS are produced by subjecting the spores of *Lycopodium clavatum* (commercially purchased) to a series of treatments with food or medical grade acids, alkalis, bleaching agents and ethanol.

9. Referring to a number of reviews, the applicant states that SS are highly stable and are resistant to a number of agents (various acids, alkalis, organic solvents and enzymes), and that they have been shown to pass unchanged through the gut.

***Discussion**:*

At the Committee's request, the applicant provided further details on the production process, including a diagrammatic representation of the process and details of all reagents used. The applicant also explained that sporopollenin is extremely resistant to treatments in the process, ensuring that other contaminating materials such as nitrogenous material are completely removed, leaving sporopollenin as the sole component.

The Committee was satisfied with the production process on a laboratory scale but requested further information on how this will be scaled up for commercial production. The applicant referred to advice from a major ingredient company that its process is capable of being scaled up for bulk routine commercial production. The Committee noted that species of club moss are becoming

endangered in some parts of the world and the applicant provided information about sustainability of the source material. The Committee was satisfied that all its concerns had been addressed and no further information was requested.

III. History of the organism used as a source of the novel food

10. The applicant refers to food safety regulatory approvals for sporopollenin shells in N. America, Asia and Australasia, but no further details are provided in the dossier. The applicant does not refer to any history of consumption of club moss or of any products derived from it.

***Discussion**: The Committee did not raise any concerns relating to this section of the dossier.*

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

11. The applicant foresees that their ingredient could have widespread application as a carrier for oils, for example to encapsulate fish oils or other DHA and EPA-rich oils, and for other minor ingredients such as vitamins. They are therefore seeking authorisation for the use of SS in the following foods:

    - food supplements,
    - dietary foods for special medical purposes,
    - foods for use in energy-restricted diets for weight reduction,
    - other foods for particular nutritional uses as defined in Directive 2009/39/EC,
    - fine bakery wares,
    - breakfast cereals,
    - dairy analogues
    - dairy products.
    - non-alcoholic beverages (including dairy analogue and milk-based drinks),
    - spreadable fats and sauces (dressings).

12. It is anticipated that SS filled with oils will primarily be consumed as food supplements, as the resulting product is a free-flowing powder that the consumer can ingest as preferred, e.g. stirred into a drink or yoghurt or sprinkled on breakfast cereal. The applicant provides intake estimates based on the potential use of SS as carriers for oils added to bread and pasta, two of the most likely applications of their ingredient in manufactured foods.

13. The applicant has estimated the potential consumption of SS on an all consumer basis by the 95th percentile UK consumer of bread and pasta. This is a worst-case scenario, assuming that all the food consumed in these categories was a functional food containing the novel ingredient. Estimates can be found in the table below:

Product categories	All consumers 95th percentile (g/person/day)	SS % inclusion	Grams of SS consumed per day
Bread	206.3	0.005	1.0
Pasta	63.7	0.005	0.3
Bread and pasta combined	215.9	0.005	1.1

Note: high level consumers of one food are unlikely to be high level consumers of another and hence the 95th percentile values for bread and pasta are not additive.

14. The applicant states that SS are not predicted to be a risk for any specific population group and is not intended to replace any existing foods in the diet.

15. The applicant highlights that SS can be filled with an active ingredient on a weight to weight ratio (shell:ingredient) between 1:1 and 1:4. Using fish oil as an example, a ratio of 1:1 is optimal (Barrier et al., 2010) so the weight of SS consumed is the same as the active ingredient. Higher loading would result in lower SS consumption to deliver the required dose of fish oil. The applicant mentions that normal supplemental intake of cod liver oil (rich in omega-3) is 0.5-1.5g/day, therefore, consumption of SS-based cod liver oil supplements would result in an equivalent intake of SS i.e. 0.5-1.5g per day.

16. The ACNFP Secretariat has made a further intake estimate based on the types of foods that DHA + EPA rich algal oils are authorised to be added to in the EU. Based on NDNS[1] data, the greatest 97.5th percentile all-user intake of DHA+EPA is approximately 1.7g per day. According to previous novel food applications DHA+EPA combined make up approximately 30% of the oil, and therefore approximately 5 g of oil would be consumed in total. Using the 1:1 SS to ingredient ratio above, this would equate to a daily intake of 5 g of SS.

Discussion*: The Committee's only comment on this section related to existing exposure to sporopollenin from mushrooms. The Committee requested further information to support the comparison between sporopollenin from club moss and components found in edible mushrooms. The applicant stated that there are many references to show that sporopollenin is present within the exine walls of*

[1] UK National Diet and Nutrition Survey

fungi and referred to genetic evidence showing that the chemical composition and structure of sporopollenin in mushrooms is very similar to land plants such as L.clavatum. The applicant therefore concludes that there is a close comparison between sporopollenin of the shells of club moss and fungi walls, including those of the exines of edible mushrooms. The Committee was satisfied with the applicant's response and no further questions were raised relating to this section of the dossier.

XI. Nutritional information on the novel food

17. The applicant states that sporopollenin is resistant to acid and enzyme digestion and does not itself have any nutritional value, passing unchanged through the digestive tract.

18. The applicant refers to clinical studies which have shown that certain ingredients incorporated within SS (ethyl ester of EPA and vitamin D) are absorbed more rapidly than when taken alone.

19. The applicant states that there is no evidence of adverse health effects resulting from consumption of sporopollenin shells.

Discussion:

The Committee requested further information on the carrier properties of the shells and mechanisms of ingredient release. The applicant outlined different possible ways in which the contents of SS are released. Factors that are important in facilitating release of the contents include gut peristalsis, bile acid and pH.

The Committee also requested more information relating to the ingredients intended to be encapsulated into SS. Data submitted by the applicant indicated a 2.2 fold increase in bioavailability of encapsulated vitamin D and the Committee raised concerns about the effects of encapsulating other ingredients and possible safety implications. The applicant stated that it has investigated omega-3 oils, vitamins and polyphenols; and it is intended that these are the materials that would be encapsulated for food use in the first instance. However, the applicant would be interested to encapsulate and deliver iron (II) and probiotics at a later stage, as well as other substances.

The applicant states that manufacturers will be made aware of the extent to which bioavailability can be enhanced using SS, so that the content of encapsulated components can be adjusted and an overdose of such a vitamin, omega-3 oil or polyphenol can be avoided. The applicant stated that, an advantage of SS is that the active substances are simply physically absorbed in

the encapsulation process and then released during passage through the GI tract. In relation to the increased bioavailability of vitamin D1, the applicant pointed out that this would not pose a risk of exceeding the recommended daily dose of this vitamin.

Members were content that there were no outstanding safety concerns relating to SS themselves, but some concern remained about the consequences of inclusion in SS of some ingredients , such as products containing protein, including probiotics (where encapsulation could protect allergenic proteins from digestive enzymes) and substances where a change in bioavailability could have significant consequences (such as iron). The Committee therefore advised against the use of SS to encapsulate allergenic materials. The Committee also considered that the allergenic properties of encapsulated proteins are unpredictable and therefore advised against the incorporation of proteins into SS. In other cases, manufacturers should evaluate carefully the potential for formulations to alter the bioavailability of ingredients where this could affect the consumer adversely.

XII. Microbiological information on the novel food

20. The applicant provided certificates of microbiological analyses which demonstrate that no detectable microbiological contamination (Enterobacteriaceae, yeasts and moulds, *E. coli*, coliforms and *S. aureus*) was found in samples of SS up to three years old.

Discussion*: The Committee did not raise any concerns relating to this section of the dossier.*

XIII. Toxicological information on the novel food

21. The applicant emphasises that SS are inert and studies have demonstrated that they pass unchanged through the body.

22. The applicant highlighted an animal feeding study where CD1 mice were administered SS by oral gavage. In this study, three groups of mice were dosed by gavage with empty SS (1.44mg) or the same amount of SS filled with vitamin D2 or EPA ethyl ester. No adverse effects were noted and no SS were observed in animal tissues at autopsy, though SS were observed to be lodged in the microvilli of the intestine. SS were not detected in urine, but intact SS were recovered unchanged in the faeces, visible by microscopy. Vitamin D-loaded SS contained only trace quantities of vitamin D upon recovery. The study also showed that an increase in quantity of faecal excretion and faecal volume was observed which the applicant attributes to dosing with a large volume of SS.

23. The applicant summarised a human study where two volunteers each consumed 5 g of SS. Urine was collected over a 12 hour period and analysed for SS, and no SS were recovered during this period.

***Discussion**: The Committee requested additional data from the applicant on this section of the dossier.*

The Committee expressed concerns about the implications of SS being lodged in intestinal villi, as was reported to occur in mice. The applicant stated that, while sporopollenin shells were found close to the intestinal wall villi in mice culled after 12 h, no shells were found close to intestine tissue walls in samples collected after 24 h. This observation suggested that the shells had been removed from the intestine and excreted in the faeces.

The Committee sought evidence to support the applicant's statement that 100% of ingested SS are egested unchanged, minus their contents, including information on the suitability of the detection limits used to test for the presence of SS in urine, faeces, blood and gut.

The applicant highlighted that the probability of finding SS in any tissue or bloodstream is extremely remote. The exines are approx. 30 micrometres in diameter and there is much literature indicating that particles >3 micrometres are too large to cross the GI tract via enterocytes, M cells or Peyer's patches. The likelihood of SS being digested at any point during passage through the GI tract in humans is extremely remote, given their stability. The applicant provided additional summary data from the mouse study, including measurements taken over 24 h rather than 12 h, and using an alternative counting method (Fluorescent Activated Cell Sorting or FACS) to replace the use of a haemocytometer. These data confirmed that that SS in faeces contained none of the materials that were initially loaded into them. Scanning electron microscopy also showed that the shells appeared completely unchanged in size and morphology. Recovery of SS in faeces was between 86±3% and 93±3%. The applicant acknowledges that these values are short of 100% but attributes this to loss during handling or the possibility that more exines could have been egested after a further period. The Committee agreed that the reported recovery is normal for this type of study.

To demonstrate the suitability of the methods and detection limits used to test for the presence of SS in urine, blood and gut, the applicant drew the Committee's attention to two important features of SS; their unique morphology, which enables their detection and counting by microscopy, and their highly fluorescent nature. The applicant therefore concluded that haemocytometry and FACS are suitable techniques for the detection and enumeration of SS.

In addition to the studies described in the dossier, the applicant also submitted results of extended human studies, using additional volunteers to investigate the presence of SS in urine and blood at various times after ingestion. SS were not found in either blood or urine. The applicant also attempted to investigate the presence of SS in gut sections from mice but counting (by fluorescent light microscopy) was extremely difficult and the measurements were not reproducible. The applicant also reported that scanning electron micrographs were obtained using a gold sputtering technique, which is capable of identifying SS clearly and without ambiguity. These confirmed that SS that were present in mice faeces and gut sections were undamaged. The applicant noted however that this technique is purely qualitative and is unsuitable for use as a counting technique.

The Committee was content that the additional data provided by the applicant provided the necessary reassurance in terms of safety of SS themselves. The Committee retained some concerns relating to the consequences of inclusion in SS of some of the types of ingredients; these concerns are discussed in the discussion section of Section XI.

Allergenicity and labelling

24. Although the applicant did not make reference to allergy to club moss, the issue of allergenicity to SS is addressed in the dossier. The applicant states that SS are extracted using concentrated mineral acid and alkali, which are known to denature and destroy proteins**.**

25. The applicant has carried out a number of protein assays (MALDI-ToF and CID-MS/MS, the Lowry method, SDS-PAGE with Coomassie staining, Bradford assay). Nitrogen analysis was also carried out. All these assays confirmed the absence of detectable protein.

Discussion:

The Committee enquired about the allergenic potential of the source club moss, including concerns relating to possible inhalation-related allergy if the product is marketed as a dry powder.

The applicant stated that any low level allergens that may be present in the starting material are removed during the harsh conditions employed to produce SS, and has highlighted that no proteins were detected in preparations of SS using a range of detection methods acceptable to the ACNFP.

The applicant also noted that a number of human trials (66 in total, to date) have been conducted where all volunteers were requested to report any side

effects or allergic reactions to SS within 48 hours of taking SS; no such reports occurred.

Supporting evidence is also available from the widespread consumption of other sporopollenin-containing substances such as pollen and spores; however, the Committee did not regard this to be relevant for the purposes of this assessment.

The applicant also noted that L. clavatum spores are sold on the open market (although not specified where) as a herbal remedy and according to "pollenlibrary.com" the spores are regarded as a moderate allergen http://www.pollenlibrary.com/Specie/Lycopodium+clavatum. However, the applicant reported that there is relatively little peer reviewed literature reporting on the allergenicity of L. clavatum spores, other than one isolated report of occupational asthma (Cullinan et al., 1993).

The Committee emphasised that pollen is a potent vehicle for allergens and it is therefore important that SS are not used as carriers for proteins, which could evade digestion and potentiate uptake and presentation to the immune system when loaded into the shell and subsequently initiate an allergic reaction in a susceptible individual (see Section XI above). The applicant provided results from an in vitro study which shows that both insulin and lysozyme loaded into sporopollenin shells are released rapidly in simulated gastric fluid (approx. 96% release after 5 minutes and completely released after one hour). The applicant therefore concluded that sporopolleinin shells from Lycopodium clavatum have a very low affinity for insulin, lysozyme and very probably other proteins and peptides, and confirmed that they do not intend SS to be used as a carrier for proteins.

The Committee sought further reassurance about the effects that these small particles may have when they are in a food matrix, in terms of inhalation of the powder and effects on the gut and immune system. The applicant noted that is generally recognised that most particles with a diameter greater than 10 µm (one third of the average size of SS particles) are deposited in the nose or throat and do not penetrate the lower tissues of the respiratory tract. The applicant acknowledged that the product is oven dried to give a fine mono-dispersed powder but noted that the exines are demonstrably free from any potentially allergenic proteins. The applicant stated that SS resemble starch in terms of particle size and lack of protein, and the procedures used in the food industry to control airborne starch particles should be appropriate for handling SS. Members noted that deposition in the nose and upper respiratory tract is crucial in the ability of pollen grains and spores to cause allergic reactions and

this provides an additional reason for not combining SS with allergens and other proteins.

CONCLUSION

The ACNFP has completed its assessment of SS to encapsulate functional ingredients and did not identify any significant safety concerns relating to this ingredient. On request, the Committee received further information from the applicant on several aspects of this application:

- Specifications
- Production process
- Nutritional information
- Intakes
- Toxicological information; including the fate of SS
- Allergenicity
- Sustainability

After reviewing the applicant's responses to these issues, the Committee did not have any outstanding safety concerns relating to sporopollenin shells per se.

However, some concern remained relating to certain types of ingredients that could potentially be incorporated into the shells such as allergenic ingredients and ingredients where a small change in bioavailability could have significant consequences. The Committee therefore recommended that:

(a) SS should not be used to encapsulate known allergens;

(b) Manufacturers should carefully evaluate the potential for new formulations to alter the bioavailability of ingredients carried within and delivered to the body from the shells, where this could affect the consumer adversely[2]; and

(c) SS should not be used to encapsulate proteins

The ACNFP therefore concluded that, subject to these recommendations, sporopollenin shells meet the criteria set out in Article 3(1) of Regulation (EC) No 258/97, namely they do not:

- present a risk to the consumer

[2] The Committee is considering whether manufacturers' controls are likely to be sufficient to address this recommendation and would welcome any comments during the public consultation on this draft opinion

- mislead the consumer
- differ from foods or food ingredients which they are intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer.

DRAFT May 2014