COMMITTEE PAPER FOR DISCUSSION **ACNFP/113/9**

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

SPOROPOLLENIN SHELLS FROM CLUB MOSS

ISSUE

The Committee reviewed this application for the first time in February of this year and requested further clarification on a number of points. The Committee is now invited to consider the applicant's response and to indicate whether its outstanding questions have been addressed.

Background

1. 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 only of carbon, hydrogen and oxygen.

2. Sporopollenin shells are produced by emptying spores of their genetic, lipid and protein material to leave an empty sporopollenin shell. The applicant's intention is to fill the empty shell with most likely, functional ingredients such as fish oils or vitamin D. Sporopollenin shells will therefore function as a novel system to deliver functional ingredients into the body. The novel ingredient plus its contents makes a powder which could be incorporated into food or drink by the consumer or manufacturer.

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

4. This application by Sporomex Ltd., a UK based company, for authorisation of sporopollenin shells from club moss (*Lycopodium clavatum*) as a novel ingredient in the EU was reviewed for the first time by the Committee at its meeting in February 2013 (ACNFP/109/7). The Committee requested further information on a number of issues: production process, nutritional information, toxicology, allergenicity, intakes, sustainiblity and specifications. A letter outlining the Committee's request for additional information was sent to the applicant on 12 March and is attached as **Annex A**. The applicant has provided responses to the Committee's questions, the full response is attached as **Annex B**. Further details on the applicant's mouse feeding studies (unpublished) are

attached as **Annex C. Annexes B and C must be treated as Protect: Commercial.**

Summary of the applicant's response

Production Process

5. The Committee requested further details on the production process, including a diagramatic representation and information on the effects of the production process on the novel ingredient. The Committee considered it unlikely that ALL internal contents of the shells are removed as suggested by the applicant and requested information on the components present in the material at the end of processing.

6. The applicant has provided further details on the production process (Protect-Commercial), attached in Annex B, p 8-11. A diagrammatic representation is presented, including details of all reagents used. The applicant also explains that sporopollenin is extremely resistant to treatments in the process, ensuring that other contaminating materials such as nitrogenous material are removed from sporopollenin, leaving sporopollenin as the sole component.

Nutritional Information

7. The applicant has provided information to demonstrate that sporopollenin shells are not of any nutritional value. An *in vitro* study is summarised which shows that sporopollenin shells (from another source) are resistant to digestion or degradation when treated with simulated human gastric fluid containing proteases at pH 1.5 and viewed by SEM (scanning electron microscopy). The applicant states that its own *in vitro* studies using a similar array of digestive enzymes also support these findings, but no further details are provided.

8. A mouse feeding study is also described, where *Lycopodium clavatum* sporopollenin shells (1.44 mg, source unconfirmed) were ingested by mice and egested faecal sporpollenin shells were examined to evaluate the extent of erosion or degradation. The applicant states that the study showed that none of the sporopollenin shells showed signs of erosion or degradation on comparison of close-up images of the shell surfaces before and after ingestion (Annex B p11-13). The same study also showed that exines were not detected in urine samples, liver, kidney, lung or intestine tissues in the 400 sections investigated (Annex B appendix 2, full details in Annex C).

9. The applicant concludes that a significant proportion of ingested sporopollenin shells pass through the GI tract. To note: the mouse feeding study refered to by the applicant showed that approx. 65% of ingested sporopollenin shells were recovered in faeces at 12 hours after ingestion.

Toxicology

10. The Committee did not feel sufficient data had been provided by the applicant and requested a full study report of the rodent feeding study. The applicant informed the Secretariat that these data are as yet unpublished and has provided a document to further expand on the mouse feeding studies (Annex C).

11. The Committee requested further information from this study on the fate of the shells in the GI tract, as the electron micrograph supplied in the dossier did not provide a large amount of information and could not be used as evidence that spores pass through the GI tract unchanged. The applicant has provided images generated by light microscopy and scanning electron microscopy illustrating that sporopollenin shells pass through the GI tract with no erosion or degradation (Annex B p14 and Annex C).

12. In addition to the mouse feeding study, two trials were also conducted in human volunteers (Annex C). In the first, sporopollenin shells (200 mg), prepared in accordance with the method in this application, were ingested by two sets of six human volunteers using two separate shell preparations. No shells were detected in their urine over a period of eight hours. The same technique was used to determine the number of shells (400 mg) migrating into the bloodstream following oral ingestion with milk; no shells were detected. None of the twelve human volunteers reported any adverse effects from taking the sporopollenin shells.

13. The Committee requested further information on the carrier properties of the shells, in terms of ingredient release for example. The applicant has outlined different possible ways in which the contents of sporopollenin shells are released. Factors important in facilitating release of shell contents include gut peristalisis, bile acid and pH (Annex B, p15-16).

14. The Committee expressed concerns about the implications of sporopollenin shells being lodged in intestinal villi, as was reported in the dossier, and further information was requested on the implications of this observation. The applicant states that, while sporopollenin shells were found close to the intestinal wall villi in mice culled after 12 h as shown in Figures 3 a and 3b (Annex B, p 13 and Annex C), no shells were found close to intestine tissue walls in samples collected at the 24h point of the study. The observation suggests that the shells had been removed from the intestine walls and excreted in the faeces.

Allergenicity

15. The Committee emphasised that pollen is a potent vehicle for allergens and as such, it is important that sporopollenin shells are not used as carriers for proteins, which could remain intact within the shell and subsequently initiate an allergic reaction. The Committee also enquired about the allergenic potential of the source club moss, including concerns relating to possible inhalation-realted allergy if the product is marketed as a dry powder.

16. The applicant has provided evidence that sporopollenin shells have a very low affinity for proteins. An *in vitro* study has been conducted to illustrate that insulin or lysozyme loaded into sporopollenin shells are released rapidly in simulated gastric fluid (approx. 96% release after 5 mins and completely released after one hour). The applicant therefore concludes that sporopolleinin shells from *Lycopodium clavatum* have a very low affinity for insulin, lysozyme and very probably other proteins and peptides.

17. The applicant also states that any low level allergens that may be present in the starting material are removed during the harsh conditions employed to produce sporpollenin shells, and has highlighted that no proteins were detected in preparations of sporpollenin shells (it is highly probable that these data relate to sporopollenin shells produced using the applicant's method, but this has not been explicitly stated) using a range of detection methods acceptable to the ACNFP (Annex B, p18).

18. The applicant also summarised a human patch test experiement where its sporopollenin shells were applied to the skin of the upper outer arm of six healthy human volunteers for a total of eight hours; no skin reaction was observed.

19. The applicant notes 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 reports 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 paper is attached at the end of Annex B.

Intakes

20. The applicant's dossier refers 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 states that there are many references to show that sporopollenin is present within the exine walls of fungi and refers 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.

Sustainability

21. The applicant has provided information to demonstrate that *Lycopodium clavatum* spore production appears to have been sustainable over many years and continues to be cultivated successfully on a commercial scale in Russia, China and Nepal (Annex B, p19-20).

Specifications

22. The Committee mentioned that the novel ingredient lacks a specification and requested that this be provided.

23. The applicant has addressed various parameters that are likely to be included in a specification for the novel ingredient (sporopollenin composition, protein content, pesticides, heavy metals and other contaminants, ash, chemical and physical surface properties, particle size distribution) and provided experimental data on the corresponding levels or values obtained for each parameter. However, the applicant has presented these data as experimental data and, while providing a useful indication of what may be anticipated in a specification, are not presented as a specification *per se*. The applicant has agreed to work with the Secretariat to provide a formal specification for the February 2014 meeting.

COMMITTEE ACTION REQUIRED

24. The Committee is asked whether the additional data provided by the applicant have addressed its remaining questions (bearing in mind detailed specifications will be submitted in due course).

25. If so, the Committee is asked whether it is content to recommend approval of Sporomex Ltds sporopollenin shells.

26. If not, the Committee is asked to indicate what additional data would be required.

Annexes attached:

Annex A- Letter of 12 March sent to the applicant

Annex B- Applicant's response

Annex C- Further details on mouse feeding studies.