Meeting

Krill Protein Discussion Paper

Committee Paper for Discussion - ACNFP/157/07

Advisory Committee for Novel Foods and Processes

Application for Authorisation as a Novel Food for Krill Protein Hydrolysate.

Application number RP1290

Issue

An application has been received under the novel food authorisation process (regulation 2015/2283 as repatriated) for Krill Protein Hydrolysate. The Committee is asked to advise on whether the available data provides an adequate basis for a risk assessment, and whether the novel food is safe and not nutritionally disadvantageous under the proposed use and use levels.

Background

1. On the 30th September 2021, the FSA received the submission for Krill Protein Hydrolysate from Aker BioMarine.

2. The novel food ingredient is made by a proprietary and confidential method of manufacturing krill meal (full fat and defatted) via physical processes and the addition of ethanol for extraction of fats for the defatted version of the krill meal. The hydrolysis of the krill meal uses food-grade proteases, forming the hydrolysate final product.

3. The applicant proposes to use the novel food NF in food supplements at a maximum of 25g/day, and a range of foods in order to provide an alternative to plant and animal-based proteins that are currently available as conventional foods for the same purpose.

4. The application dossier is attached as Annex A and the annex to the dossier is attached as Annex B. Both annexes contain confidential information.

This application

Identification

5. The novel food, with the trade name KPH, corresponds to Krill Protein Hydrolysate, commonly referred to as partially hydrolysed protein isolate derived from Antarctic krill (*E.superba*), and is composed of ≥85% to ≥90% crude protein on an g/100g as-is and dry matter basis, respectively. The remaining 10-15% consists of moisture, fat and predominantly ash from the use of a sodium hydroxide pH regulator.

6. This protein isolate is made by a proprietary method whereby harvested Antarctic krill are processed to either Antarctic krill meal or partially defatted Antarctic krill meal prior to treatment with a food-grade protease to produce KPH. The final composition is verified by the compositional analyses required by the FSA (Annex A [Tables 2.c.1-5] – **confidential**).

Production Process

7. The initial step in the production process involves the manufacturing of Antarctic krill meal (full fat and defatted) whereby whole Antarctic krill are harvested from the Southern Ocean and processed using physical processes.

8. The second distinct production stage concerns the hydrolysis of the krill meal to product the KPH. The addition of a food-grade protease to allow an enzymatic hydrolysis reaction to occur produces the final KPH product.

9. The production method is described and also presented in a flow diagram format (Annex A: p14 – 23 dossier [Figures 2.b.1.3.1-2 and 2-1] and Annex A for facility accreditations). Additional details on the manufacturing process were provided by the applicant upon request for further detail and clarification (Annex C, p1 – 3 RFI Letter).

10. The acceptance criteria for the raw materials and processing aids are provided (Annex B). The applicant confirms that the production facility is registered with the NSF international authorities, USA 77041/2017, and the novel food is produced under GMP by the Danish Competent Authority (Annex A – p4-5).

Composition and Specification

11. The applicant has reported analytical data for a five batches independent batches of Krill Protein Hydrolysate (defatted) and four batches of KPH (full fat). The data indicates that the manufacturing process results in a consistent final ingredient that meets the proposed specifications for the novel ingredient. All compositional analyses can be found within Annex B in folders Annex A, C and D, respectively.

12. The applicant has reported results for a proximate analysis of nine representative batches of the novel food. These results confirmed that the proximate analysis demonstrated that KPH exhibits acceptable batch to batch variation and that all components are accounted for (Annex A: p25 dossier – Table 2.c.1.1-1).

13. The applicant reported the content of solvent residues from four batches of the novel food. These results confirmed ethanol residues were present and varied from <10 – 12mg/kg (Annex A: p33 dossier). The results from all batches are below the specification limit for ethanol in the novel food.

14. The applicant has reported results for the microbial content in nine batches of the novel food ingredient – (Annex A: p38 dossier – Table 2.c.2.3.1-1). The results confirm that total plate count, and yeast and moulds, were below the specification limits besides one batch showing a mould result of 30CFU/g. A coliform result of 90 and 40 CFU/g was detected in two batches. E. coli was reported as <10CFU detected in 1g and Salmonella was not detected in 25g.

15. The applicant has reported the heavy metal content in nine batches of the novel food ingredient – (see Table 2.c.2.3.2-1). These results are below the EU permitted limits for crustaceans and fishery products: cadmium <0.5 mg/kg; lead <0.5 mg/kg; mercury < 0.05 mg/kg (Annex A: p39 dossier).

16. The applicant has reported results for dioxins, PCBs and PAHs in nine representative samples of the novel food – See Annex A – p39-40 dossier. The results confirmed that for all three of the compositional parameters above, the novel food falls well below the limits for dioxins, PAHs and PCBs as established in Commission Regulation (EC) No 1881/2006 for muscle meat of fish and fishery products and products thereof which includes crustaceans (muscle meat from appendages and abdomen).

Stability

17. The applicant reports the recommendation that KPH should be stored at <25°C in packaging under dry conditions; with a shelf life of at least 12 months. The results from an ongoing stability study, where evidence of three batches of KPH from September 2019 that were analysed from December 2019 – December 2020 was provided. The overall consensus was that the batches showed no significant changes in physiochemical, biochemical and microbiological stability but water and moisture activity increased with time (See Annex B [Annex F] – Confidential).

18. The reliability of the results from Annex B [Annex F] and the use of a non-GMP certified sample storage site was heavily queried by the Secretariat in Annex C – RFI letter. The applicant stated in Annex B – [Annex F] that “a site carrying out storage of stability samples is not required to be GMP certified for this activity in Ireland” and this was questioned. The applicant responded in Annex D – RFI letter – p4-5 that an updated Annex B [Annex A and F] provided additional answers on this matter in which GMP/GDP is no longer required, albeit an additional audit was carried out in 2022 to ensure compliance.

19. The stability of three batches of the novel food were assessed for 12 months in a stability study at 25°C and 60% relative humidity (Annex B [Annex F]). Data concerning the physicochemical properties, biochemical properties and microbiological properties were reported. The applicant states that the results meet the specification limits and demonstrate the novel food is stable under these conditions (Annex A: Table 2.c.3.2-1, Annex B [Annex F]).

12. The data shows that Krill Protein Hydrolysate is stable over this time period in terms of the physicochemical, biochemical and microbiological properties which meet the specification limits (Annex A p41 and B [Annex F]).

21. The stability data in Annex B [Annex F – Table 6 and Figure 10] however highlighted the presence of enterococcus, but the applicant had stated elsewhere in Annex A that the test method being utilised was not specific for enterococcus testing. The applicant quoted that “Concerning the observed activities of enterococcus it is noted that these results are not reflected in activities of E. coli or coliform bacteria. Moreover, the laboratory performing the testing of microbiological parameters is not accredited for quantification of enterococcus, resulting in some uncertainty of the specificity of the test.”

22. This was further queried in Annex C – RFI letter p2, in which the applicant responded in Annex D – applicant's response p6 with misinterpretation being the main reason for the discrepancy and this being modified since the report was written.

23. The applicant also states that to date no studies have been conducted to evaluate KPH stability in food matrices. The applicant expects stability to be analogous to other protein hydrolysates (see Annex A - Section 2.e.1.2 – p50).

History of use

24. The applicant provides a literature background on the source material itself, covering the history of krill consumption and cultural patterns across the globe.

25. The closely related novel food applications for Antarctic krill oil, was authorised and established in the Commission Implementing Regulation 2017/2470 (See Annex A – Table 2.e.1.1-1. For further detail). The applicant provides evidence of products currently on sale which demonstrate consumption rates of Antarctic krill.

26. The only difference in the specifications between the two substances is the phospholipid content where the lipid extract from Antarctic krill contains 35% to not more than 60% phospholipids, whilst oil rice in phospholipids from Antarctic krill contains at least 60%. The permitted uses are the same, however.

27. The applicant states that the novel food has no history of consumption or marketing in the UK (Annex A: p58 dossier). However, the applicant references the consumption and marketing of protein hydrolysates derived from alternative source materials and the evidence of this in the EU and UK. Reference has been made to egg membrane hydrolysate and rapeseed oil protein hydrolysate (Annex A – see Table 2.e.1.2-1.).

28. However, the applicant independently has gained recognition for KPH as a GRAS ingredient in the USA based on the procedures for use as a protein alternative in beverages and bars. This is at maximum levels ranging from 5-10% of the ready-to-eat products, whilst protein powders for beverage formulations will vary up to the maximum of 90% by weight in powder (Annex A: p51 dossier). Further clarification of this statement was sought in Annex C – RFI letter p2 in which the applicant responded in Annex D – Applicant's response – p7 by providing a GRAS notice document in Annex B – [Annex G].

Proposed uses and Intake

29. The applicant states that the novel food is intended to be used by active adults and elderly individuals; it will not be targeted towards infants or young children, and pregnant or lactating women. There are no other precautions or restrictions to use of Krill Protein Hydrolysate (Annex A – p53 dossier).

30. However, further clarification was sought on the use of the novel food in adolescents as the applicant stated elsewhere that the NF was marketed for those in the general population and not children up to 3 years; yet claimed the target was for active adults/elderly only (See Annex C – RFI letter p2). See Annex D - RFI response p7 for clarification: 'Products containing Krill Protein Hydrolysate are intended to be consumed by the general population above 3 years, excluding pregnant and lactating women'.

31. The novel food is intended for use as an alternative protein source in a range of foods and beverages, as well as food supplements. Krill Protein Hydrolysate is not intended for use in infant formula and follow-on formula. These conditions of use reflect the currently permitted use for rapeseed protein as outlined in the EU Union list, established under Regulation (EU) 2017/2470.

32. The applicant states that krill protein hydrolysate in food supplement form (25g/day) are neither intended nor expected to be used in conjunction with other foods with added krill protein hydrolysate on the same day (see Annex A - p57 dossier). For purposes of conducting an exposure assessment (see Section 2.f.3 and below), the applicant has provided representative food uses and use-levels for the NF. Uses include bakery, cereal bars, mineral waters and meal replacement dietary products.

33. The applicant concludes that in reality the novel food will present itself as an alternative protein source in foods – akin to soy protein isolates and non-meat proteins already utilised within the UK.

34. The applicant reports their estimated exposure rates using the EFSA comprehensive database and FoodEx2 food categorisation system – Annex A: p53 dossier Table 2.f.2-1. and Annex B [Annex G]. Alongside this, the applicant has conducted their own confidential exposure assessment. Estimated dietary exposure calculations were calculated on a per person/per kilogram body weight basis and covered each age category as well as exposure in conventional foods and food supplements (see Annex B [Annex G])

35. Applicant states that exposure to krill protein hydrolysate via conventional foods rarely exceeded 0.1g/day but ranged from 0.1-0.6 g/day on average for other children, adults, elderly-very elderly at high-level doses at P95. An estimate of 0.9g day at P95 was observed for adolescents. The novel food when taken via food supplements (at 25g/day) ranged from 329mg/kg bw/day in the elderly (76kg) to 1082 mg/kg bw/day in other children (23.1kg). Applicant estimates that the main contributors to Krill protein hydrolysate intake would be ‘cereal bars’ and ‘protein products.’

36. The applicant considers the uncertainties which have arisen from their estimate assessment which may contribute to the additional exposure of the novel food via chronic consumption or combined intake. It was concluded that chronic consumption of foods containing the novel food by infants and toddlers have the highest magnitude of uncertainty. As a result, the applicant intends for the novel food to replace plant and animal derived protein products and so, concludes that they will ‘contribute to’ not ‘alter’ daily protein intakes by the UK population.

37. Applicant provides reassurance of their above statement. Supplements containing Krill Protein Hydrolysate will be clearly labelled with maximum daily doses and a cautionary statement which states that ‘other foods containing Krill Protein Hydrolysate should not be consumed on the same day’.

Absorption, Distribution, Metabolism and Excretion (ADME)

38. A statement provided by the applicant states that as ‘the novel food is primarily composed of protein and contains an amino acid profile that is comparable to that of krill meal, it is assumed that the novel food will be processed via normal physiological processes in a manner similar to other dietary proteins’ (Annex: p59 dossier). The applicant concludes that this statement supports the ADME portion of their dossier and as such meets the application requirements to ensure safe absorption and distribution of the novel food in consumers (Annex A: p44 dossier).

39. The applicant provided a literature review of a number of published studies ranging from 2007-2017 where studies by Miner-Williams et al (2014) and Vahdatpour et al (2016) formed the basis for the review in particular (Annex A: p59). The results discuss the normal physiological processes of protein digestion and compares them to the assumptions made regarding the digestion of the NF. It

can be concluded that the evidence provided indicates that regardless of the protein source consumed, the human circulation would encounter mostly individual amino acids and a small amount of short di- and tripeptides.

40. The applicant reports the results from a recently published study on the bioavailability of Krill Protein Hydrolysate which established that the novel food ingredient has the amino acid availability following digestion that is comparable to whey and soy protein isolates (see Section 2.h.5.1.). Further clarification was sought regarding this study as it was conducted at Aarhus University in Denmark, stating compliance with research ethics yet no evidence of GLP or CoA had been provided (see Annex C, p7 – 8 RFI Letter and Annex D – Applicant's response). See also Annex B [Annex L] which shows the GCP approval and the Danish Act ethical approval certificates for this particular study.

41. The applicant provided additional supporting evidence from a published study (Delaney et al, 2008) on the digestion processes of proteins in the gastrointestinal tract in humans, stating further that digestion via denaturation and degradation pose a less likely safety concern (Annex A: p59 dossier).

Nutritional Information

42. The applicant states that the nutritional analysis of Krill Protein Hydrolysate is characterised primarily by protein (no less than 85g/100g on an as-is basis) with lesser amounts of fat (no more than 1g/100g), ash (no more than 7g/100g) and moisture (no more than 10g/100g). Additionally, the presence of anti-nutritive components related to crustaceans such as chitin are removed upon isolation of the protein fraction of KPH production. The applicant concluded that the analytical data for the representative KPH batches demonstrate that levels of protein, amino acids, vitamins and minerals are within acceptable levels and do not highlight a potential safety concern – (see Annex A – p25 – 31 dossier; Tables 2.c.1.1-4).

43. The applicant discusses the dietary reference values for protein in the EU for all age groups and compares this to the protein intake potential of the novel food (Annex A – p60-61 dossier – Table 2.h.1-1). Using published literature which discusses the tolerable upper intake levels of protein, the applicant recognises that the novel food could represent a substantive fraction of the daily protein requirements but ultimately concludes that the reported levels fall under the tolerated levels. Under the representative conditions of use, mean and high-level consumer-only intakes of Krill Protein Hydrolysate were estimated to be greatest for adolescents at 5.1 g/day and 45.4 g/day, respectively.

44. The applicant provides a comparative table which depicts the amino acid composition of the novel food in comparison to the typical values reported for other protein isolate products (whey, casein and pea) which it could potentially replace in an individual's diet. The results conclude that KPH represents a total amino acid composition of 42.6%, whereas the three comparative counterparts depict results of 43, 34 and 30%, respectively (See Annex A – p62 – Table 2.h.2-1). The novel food therefore has the potential to act as a reliable source of essential amino acids.

45. The applicant reports the results of the mineral intake potential of the novel food when assessed against the NRV levels established by the EFSA. As seen in Annex A – Table 2.c.1.3-1, there were no NRVs or ULs for aluminium, tin, and sulphur, these minerals were not assessed. The levels of aluminium and tin in Krill Protein Hydrolysate were low or below the level of detection and were not considered a safety concern. Mean levels of sulphur were 9,800 mg/kg of the Krill Protein Hydrolysate batches tested; however, based on the intended use, the levels of sulphur would be 50 and 445 mg/day at the most conservative mean and high-level intake of 5.1 and 45.4 g Krill Protein Hydrolysate, respectively.

46. The applicant also reported that at the mean level intake of Krill Protein Hydrolysate, the estimated contribution of minerals to the established NRVs was low, ranging from 0.02% for potassium to 15.08% for selenium and none of the mineral levels exceeded the ULs as established. However, for the high-level intake of Krill Protein Hydrolysate, some of the minerals would be considered significant contributors to the NRV including calcium (50.17%), magnesium (52.67%), copper (66.31%), selenium (195.20%), and iodine (61.60%). However, none of the minerals exceeded the ULs established for any of the minerals and so it was concluded that actual exposure to minerals from the novel food ingredient will be lower than estimated.

47. The applicant reports that KPH contains measurable levels of vitamins B2, B3, B8, B9 and B12 (Annex A – Table 2.c.1.4; p31 dossier). The results conclude that the novel food ingredient will not make a significant contribution to dietary vitamin intake under the intended conditions of use. Moreover, where ULs are established, none of the measurable B vitamins would exceed this level under the most conservative levels of intake of 45.4 g/day in adolescents. Thus, Krill Protein Hydrolysate is does not adversely impact vitamin intake under the proposed conditions of use.

Toxicological Information

48. The applicant was requested to produce further evidence of laboratory compliance and the ability to undertake genotoxicity testing (Annex C – RFI letter: p3). Whilst OECD 471 and GLP approved, the CoA provided but was not specific to LabCorp or genotoxicity testing. See applicant's response in Annex D - RFI response p8 – in which GLP of LabCorp and further GCP approval was answered and provided in Annex B [Annexes L and M]; Ethical treatment certification was also provided in Annex L.

49. The applicant reports the results from a collection of published toxicology literature, as a means of supplying the evidence required by applicants for successful validation and suitability of the toxicological portion of the dossier (Annex A: p83 – 87 dossier and Annex D – applicant's response to RFI – p14-18). A tiered approach was not followed for the safety assessment, rather a Weight of Evidence (WoE) approach was used. The WoE approach was utilised as the literature review indicated several relevant studies that had been previously conducted providing the necessary subchronic toxicity information.

50. The applicant further stated in Annex D – applicant's response to RFI – p15, the primary evidence of safety provided by the protein quality and amino acid composition together with the detailed compositional and impurity data on other components of Krill Protein Hydrolysate supports the safety of KPH. Safety for the intended use is corroborated by information on the history of dietary consumption of Antarctic krill and the results of studies in animals on test articles related to Krill Protein Hydrolysate. As such the use of additional laboratory animals to further add to this toxicity data was deemed to be unnecessary and a literature review sufficient.

51. The applicant reports the results from a 90-day feeding study in Hans-Wistar rats (OECD TG 408) conducted to GLP principles. The groups fed a diet of the applicant's 9.67% krill powder were reported to be well tolerated at doses up to 2,250 mg/kg body weight/day in males and 2,640 mg/kg body weight/day in females (Annex A: p83 – 84 dossier). The full study report is available in Annex B [Annex N – confidential].

52. The applicant has reported the results from a digestibility and reproductive study evaluating the effects of the novel food precursor in the mink at 1.5 g/day and 2.0 g/day (Annex A: p66 – 70 dossier). The report concludes that no adverse effect related safety concerns were raised during these trials and the novel food precursor exhibited a similar nutritional value akin to fish meal and was not associated with any adverse effects when incorporated into the diet of female mink at levels of up to 17% (the NOAEL), equivalent to around 35 g/kg body

weight/day (or 22.54 g krill protein/kg body weight/day).

Allergenicity

53. The applicant states that the novel food is derived from a crustacean and it is known other crustaceans are priority allergenic foods. The applicant has considered the well-known allergenic potential of shellfish, with additional information provided for the associated symptoms and specific sensitisation to crustaceans and products thereof which are currently listed in Annex II of Regulation 1169/2011 as substances causing allergies or intolerances.

54. The applicant has considered the relevance of this background to their Novel Food mainly through a literature review (Annex A – p.100 - 2.j Allergenicity). The applicant has provided a discussion on tropomyosin as the major allergen, identifying two supporting studies which considered krill tropomyosin and their relatively high sequence homology to those of other shellfish and crustaceans. Applicant has concluded that those with shellfish allergies should avoid krill-containing products.

55. The product contains protein at levels of no less than 85g/100g on an as-is basis (or no less than 90g/100g on a dry matter basis) – see Section 2.c.1.1 of Annex A.

56. Taking the above into account, the applicant has concluded that any foods containing Krill Protein Hydrolysate will be labelled appropriately as “contains crustaceans (Antarctic Krill) and products thereof” to warn consumers susceptible to crustacean allergens to be aware of the need to avoid krill-containing products. (Annex A: p100 dossier).

Committee Action Required

- The Committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food.
- If so the Committee is asked whether it is content to recommend approval of the novel food as an ingredient to be added to the range of foods specified.
- If not, the Committee is asked to indicate what additional data would be required.

ACNFP Secretariat

December 2022

Annexes

ACNFP-157-10-Annex A – Dossier [Confidential]

ACNFP-157-10-Annex B – Annexes and References [Confidential]

ACNFP-157-10-Annex C – Request for further information at validation phase.

ACNFP-157-10-Annex D – Response from Applicant to RFI at validation phase.