ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

DRAFT OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR *CALANUS FINMARCHICUS*

Applicant: Calanus AS of Norway

Responsible Person:

EC Classification: 2.2

Introduction

1. An application from Calanus AS of Norway for use of calanus oil, oil from the copepod (a marine zooplankton) *Calanus finmarchicus*, as a novel ingredient was accepted by the Food Standards Agency on 31 January 2011. A copy of the application was placed on the Agency's website for public consultation. Following questions from the Committee, the applicant undertook further studies to support their application, which have enable the Committee to complete their initial opinion.

2. The applicant intends that calanus oil will be marketed in the form of food supplements rich in the polyunsaturated fatty acids DocosaHexaenoic Acid (22:6(n-3), DHA) and EicosaPentaenoic Acid (20:5(n-3), EPA). The applicant foresees this oil to be a direct replacement for other polyunsaturated fatty acid rich oils, including krill oil and algal oil from *Schizochytrium* sp. and *Ulkenia* sp., for use in food supplements.

3. In accordance with the data requirements framework for novel foods, calanus oil has been classified as a complex novel food from non-GM sources source (class 2.2).

I Specification of the Novel Ingredient (NI)

4. The applicant has provided a specification for calanus oil which is detailed below. The applicant notes that the specification is consistent in many respects with the specifications for the other novel marine and algal oils. Detailed information regarding the analytical methods and laboratory accreditation is provided in Appendices A8 and B5 of Annex 1.

Proposed Specification of Calanus Oil	
Parameter	**Specification**
Appearance	Ruby slightly viscous liquid
Odour	Light shellfish
Water	<1%
Wax Esters[1]	>85%
Total Fatty Acids	>50%
EPA	>3%
DHA	>5%
Total Fatty Alcohols	>36%
C20:1 n-9 fatty alcohol	>12%
C22:1 n-11 fatty alcohol	>16%
Trans fatty acids	<1%
Peroxide value	<3 meq. O_2/kg

5. The applicant provided a detailed overview of the lipid profile of the oil which confirms that the composition is primarily fatty acid esters (>85%) The applicant did not comment on the batch-to-batch differences in the level of free fatty acids but notes that these and other minor components (phospholipids, mono-, di- and tri-glycerides etc) are present at levels no greater than 3%.

6. The applicant suggested that the fatty acid profile of calanus oil is consistent with that of other marine oils (see Table X1.2.1-1) p38). It was noted that, although each individual fatty acid is, generally, present in each type of oil, there are marked differences in the quantities. The fatty acids seen in calanus oil include DHA and EPA (5% and 7% respectively) and also stearidonic acid (SDA) (C18:4 n-3) (7%). In contrast to other marine oils, the applicant explained that the majority of DHA and EPA is present in an esterified form rather than as triglyceride. Analysis of the fatty alcohol component of the oil indicates that the major alcohols present are the monounsaturated alcohols C20:1 n-9 (13%) and C22:1 n-11 (20%).

7. The level of astaxanthin present in the oil (0.08%) is relatively high compared to other marine oils leading to its red colour. Other analyses (trans fats, heavy

[1] Wax esters are monoesters of long chains fatty acids with long chain fatty alcohols. They are found in certain fish and zooplankton and in other waxy materials such as plant waxes and beeswax.

metals) indicate that the levels present are within maximum permitted levels. Analyses of a range of environmental contaminants (dioxins, dioxin like polychlorinated biphenyls, polycyclic aromatic hydrocarbons polybrominated diphenyl ethers, acrylamide and pesticide residues) were also carried out and all were found to comply with recognised or published safe limits.

8. The protein level present in calanus oil was found to be below the limit of detection in each of two analytical methods employed. The applicant has carried out a number of stability studies which, in their view are sufficient to set a shelf life of 3yrs for the oil, under nitrogen.

Discussion: *The Committee requested further information on the dioxin and PCB levels present in the oil. In response the applicant repeated the associated analyses. Amendments were made to the production process to remove the contaminants and this resolved the problem, enabling compliance with statutory limits on polycyclic aromatic hydrocarbons (PAHs). On the basis of this further information the Committee was reassured that the level of these contaminants would be managed during production.*

II Effect of the production process applied to the NI

9. The production processes used to harvest, refine and extract calanus oil are conventionally used in the processing of other marine oils for food and food supplement purposes. Specific details of the manufacturing process are regarded to be proprietary.

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

III History of the organism used as the source of the NI

10. *Calanus finmarchicus* is a herbivorous copepod (small crustacean) which feeds on marine phytoplankton and is one of the most common species of zooplankton found in the North Atlantic Ocean. A characteristic of marine copepods is their ability to generate large lipid stores – primarily in the form of wax esters or triglycerides. In recent times, economically viable methods for harvesting have led to increasing availability of oils from marine sources other than fish (Krill Oil) and these are perceived to be a more sustainable source of polyunsaturated fatty acids.

11. The applicant does not claim that *C. finmarchicus* has a history of use as a food, but does highlight a number of documented examples of consumption on a small scale. These tended to be restricted to low level consumption by sailors and explorers, often when alternative sources of food were scarce. The small number of publications that mention the consumption of *C.finmarchicus* provide some evidence that consumption of *C. finmarchicus* is not intrinsically unsafe.

12. The applicant also postulates that the position of *C. finmarchicus* at the lower trophic level of the food chain, and their relatively short lifespan, means that they are unlikely to accumulate persistent organic pollutants. The applicant also noted that, as a source of nutrition for other marine organisms, *C. finmarchicus* contributes to the lipid profile of higher marine organisms, many of which are widely consumed by humans.

Discussion: *The Committee, while noting that environmental considerations were not a required area for authorisation under the novel food regulation, requested further information on the sustainability of using C. finmarchicus for oil production. The applicant provided a report on how sustainability for the marine ecosystem would be maintained. The Committee was satisfied with the response.*

IX Anticipated intake and extent of use of the NI

13. Calanus oil is intended solely for use as an ingredient in food supplements as a direct replacement for other DHA and EPA rich oils (typically fish oil). The applicant anticipates that consumption of calanus oil will be 1g/day (two 500mg capsules, equivalent to around 110 mg/day of EPA and DHA). Based on the European Food Safety Authority (EFSA) opinion regarding the reference intake values for n-3 and n-6 polyunsaturated fatty acids, consumers should have a daily intake of DHA and EPA of 250mg. This would be met by consuming 2.3g of calanus oil, which the applicant regards to be the maximum daily dose for the oil, Consumption of the key components of calanus oil for both expected and maximum levels of consumption are tabulated in the dossier.

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

XI Nutritional information on the Novel Food

14. Calanus oil is composed of fats and, in line with other edible oils and in accordance with Directive 90/496/EC, the figure of 9 kcal/gram will be used on nutritional labels as required. (In practice, the indigestibility of wax esters means that the available energy from calanus oil will be lower than for an equivalent amount of triglycerides).

15. The applicant views the fatty acids present in calanus oil to be typical of those seen in other fish oils and, in the case of stearidonic acid, in echium oil.[2] However, as noted above, the proportions of fatty acids present in calanus oil are different from those seen in the other oils including, although not detailed in the dossier, krill oil.

[2] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:180:0017:0019:EN:PDF

16. The applicant includes a detailed rationale for an increase in dietary intake in DHA and EPA, although a discussion of potential benefits is not directly relevant to the safety assessment of calanus oil. It is outside the scope of this evaluation to evaluate scientific evidence for potential health benefits from consuming the product. The applicant also provided an overview of the consumption of wax esters and wax alcohols in the European diet, which is largely from the consumption of two fish (Orange Roughy (*Hoplostethus atlanticus*) and Black Oreo (*Allocyttus* sp.)) that have a long history of consumption in the EU. The lipid profile of these fish shows a similar wax ester content: (95% in Orange Roughy and 91.5% in Black Oreo, compared with 87% in calanus oil) but the esters in the fish are comprised of different fatty acids, and fatty alcohols.

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

XII Microbiological Information

17. Calanus oil has low water activity and would not support the growth of microorganisms. The applicant includes a specification for the presence of microorganisms and has also provided data for three individual batches of the oil, each of which was within the specification.

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

XIII Toxicological information

Absorption, Distribution, Metabolism and Elimination

18. Wax esters are not efficiently absorbed by terrestrial organisms and no wax ester lipase exists in mammals. Mammalian lipases and carboxyl esterases can break down wax esters but the efficiency is low. The applicant acknowledges that, although *in vitro* studies using a range of mammalian digestive enzymes demonstrate complete digestion of wax esters within 24 hours (Gorreta *et al*, 2002), consumption of significant quantities of wax esters is associated with a human condition known as keriorrhea which occurs as a result of their passage, undigested, through the gastrointestinal tract and before pooling in the rectum. The applicant considers that such effects occur at levels of consumption that are significantly greater than are proposed for calanus oil.

19. The applicant points to studies carried out in rats in which the rate of incorporation of DHA and EPA is similar irrespective of whether the source is wax esters or fish oil or ethyl esters (Gorreta *et al*, 2002). The applicant concludes that the consumption of calanus oil at the proposed level in supplements would

provide reasonable systemic incorporation of EPA and DHA in humans. The applicant also refers to an unpublished clinical study, which did not reveal any of the expected symptoms of poor absorption, as an indicator that the esters are, to some extent, broken down and absorbed.

20. **Polyunsaturated fatty acids.** The applicant provided a brief commentary on the digestion and absorption of DHA and EPA which has been considered in detail in other previous novel food applications. The applicant concludes that ingested n-3 polyunsaturated fatty acids undergo near complete absorption and are either incorporated into tissue lipids, used to synthesise eicosanoids or oxidised to carbon dioxide and water.

21. **Fatty alcohols.** There are limited data on the absorption and bioavailability of fatty alcohols and these indicate limited absorption of larger (C24-34) alcohols. A 2002 study in rodents indicated that absorption of policosanol is estimated to be in the region of 10-35% with bioavailability 2-12% (Gouni-Berthold and Berthold 2002). Older studies using radiolabelled fatty alcohols indicate that they are converted into fatty acids in the GI tract and appear as triglycerides and phospholipids (Stetten and Schoenheime, 1940; Freidberg, 1976).

Discussion: *The Committee sought evidence to demonstrate that wax esters such as those found in the oil from Calanus finmarchicus were a bioavailable source of EPA and DHA. The applicant undertook a study in which 18 participants were tested with 4g of the novel ingredient (a wax ester containing 260mg EPA and 156mg of DHA) and a control consisting of 1g of ethyl ester marine oil (containing 465g EPA and 375g DHA). A cross over design with two study periods separated by a 7 day wash out period was used to assess each participant with both study materials. Blood samples were taken at timed intervals following ingestion of the test material over 72 hours.*

The applicant suggests the study provides evidence that Calanus oil was digested and absorbed in humans over a short term bioavailability study. They conclude from this study that the novel ingredient resulted in increased plasma concentrations of EPA and DHA relative to the control at three post prandial time points. The differences were not statistically significant. It was also suggested by the applicant that with no adjustment having been made for the starting levels of DHA and EPA in the test materials this provides an indication that EPA/DHA may be more bioavailable in wax esters than ethyl esters.

To support the bioavailability data generated, they have also provided additional references which they suggest indicate that the bioavailability of EPA/DHA is not lower in ethyl esters relative to other forms of marine oils including triglycerols. The applicant concluded that the levels of EPA/DHA in the bioequivalence study for Calanus oil are similar to those found for other oils. Following the additional

information the Committee agreed that consumers would not be nutritionally disadvantaged from taking Calanus oil over other marine oils.

Toxicity information

22. The safety of calanus oil was examined in rats fed a high fat diet as a model for obesity related (pre) diabetes. In this study (Larsen, 2011) male rats were fed a high fat diet, including 1.5% (770 mg/kg body weight/day) of the applicant's calanus oil, for 112 days. The results indicated that animals fed high fat diets (with or without calanus oil) had, as would be expected, significantly higher body weights than the controls given a lower fat diet, but consumption of the oil did not have any significant effect on food consumption or water intake. There were also no significant differences in the absolute and relative weights of the liver and heart and no abnormal findings were found during gross examination. The consistency of the rat's faeces was normal as was urine production.

23. The same authors fed *db/db* rats (used as a model for diabetes) up to 1.4% calanus oil supplemented with DHA and EPA for 56 days. Consumption of the oil did not have any significant effect on body weight gain, food or water intake or on plasma glucose levels. Plasma levels of free fatty acids and triacylglycerols were also not significantly different across the treatment groups.

24. A separate 13-week study was carried out to investigate the effect of calanus oil on the production of atherosclerotic plaques, plasma lipid levels and cytokine and hepatic gene expression. Rats were fed lipid rich diets containing 21% fat, 0.2% cholesterol and 1% of either the applicant's calanus oil, equivalent amounts of DHA and EPA or corn oil. All rats thrived and no adverse effects were observed, with the faeces of the rats that consumed calanus oil appearing normal. At the end of the study, EPA and DHA were significantly higher in the plasma of the rats fed calanus oil. The applicant acknowledges that while this study was not a toxicity study *per se*, the ingestion of calanus oil did not produce any adverse effects on relevant endpoints, with no reported differences in macroscopic appearance and in a range of blood plasma components.

25. The applicant also reports a human clinical study in which 15 healthy adults consumed 1-4 g/day of calanus oil, as a food supplement, over a four week period. Adverse effects were evaluated at the end of weeks two and four and blood samples were taken to check whether there were any haematological and clinical chemistry changes. There were two reports of transient nausea and occasional abdominal gas. Two subjects who were given 4g of the oil reported more frequent bowel habits, while one individual fed 2g reporting less frequent bowel habits, and a number of subjects reported an increase in 'positive' effects such as increased energy, less hunger. Changes in alanine aminotransferase and serum creatine levels were observed in two individuals but these returned to baseline levels by the end of the trial and were not regarded as clinically

significant. The applicant concludes that the consumption of calanus oils is safe at levels of up to 4g per day and is well tolerated at levels that are approximately 2-fold greater than the maximum intended use in food supplement products.

26. Toxicity information relating to fatty acid components in the oil (DHA EPA and SDA) largely summarises the conclusions of previous risk assessments for novel foods authorised under Regulation 258/97, krill oil and refined echium oil.

***Discussion**: The Committee noted that humans had a limited ability to process wax esters and questioned whether gastrointestinal side effects recorded in a clinical study were related to the composition of the oil. The applicant advised that, although adverse side effects were seen in the study in question, the number of subjects was too small to attribute these with certainty to consumption of calanus oil.*

The applicant highlighted two clinical studies that calanus oil is well tolerated. These studies showed no significant differences in the incidence of adverse effects in placebo and treatment groups. The applicant also reports that the 'Steering Committee of the Norwegian Scientific Committee for Food Safety' reviewed the smaller study as part of a review into GI disturbances in other human n-3 fatty acid studies and advised that the adverse effects were likely to be as a result of a relatively high oil intake. The applicant concludes consumption of calanus oil at the intended level of consumption (2.3 g/day) is not expected to result in adverse effects. This view was accepted by the Committee.

Allergenicity

27. The applicant has demonstrated that protein levels in the product are very low, 0.6µl/ml, and has additionally confirmed the absence of known shellfish allergens using an ELISA analytical test. As an oil obtained from crustaceans, calanus oil requires labelling in accordance with EC legislation on allergen labelling, meaning that the oil will be labelled as 'contains oil derived from crustaceans (marine zoo plankton)'.

Intolerance/Adverse effects to dietary wax esters

28. As described above mammals have a limited capacity to hydrolyse wax esters and this can result in an involuntary oil discharge from the anus (keriorrhea) and it is noted that certain individuals with pre-existing intestinal conditions and pregnant women may be at a higher risk. The primary sources of wax esters in the diet are currently restricted to a limited number of fish and their sale in the EU is regulated[3] by means of specific labelling, packaging and labelling to ensure that the consumer is aware of potential adverse effects. A 2004 EFSA evaluation of the adverse effects of 'Oilfish and Escolar' noted that the adverse effects could be attributed to wax esters and/or other oily compounds present in the fish, but

[3] (EC) 1021& 1020 /2008

that the precise dose required to trigger an adverse reaction could not be determined. In this opinion EFSA noted that consumption of 100g of oilfish or escolar could mean an intake of up to 20g of wax esters. The applicant points out that this is almost 10 fold higher than the proposed maximum level of intake for their oil.

Discussion: *The Committee considered these issues as part of the toxicological assessment detailed above.*

CONCLUSION

The ACNFP has completed its assessment of oil from *Calanus finmarchicus* as a novel ingredient for use in food supplements.

The Committee requested further information from the applicant on the following:

- The allergenic potential of the oil.
- The level of dioxins and PCBs present in the oil.
- Whether there could be sustainability concerns.
- Effectiveness of the oil as a source of DHA compared with other marine (etc.) oils.
- Whether gastrointestinal side effects recorded in a clinical study were related to the composition of the oil.

After reviewing the applicant's responses to these issues, the Committee did not have any outstanding safety concerns. Based on the evidence provided the ACNFP therefore concluded that oil *Calanus finmarchicus*, used as proposed by the applicant is unlikely to present a health risk to consumers. Based on the bioavailablilty information provided they also concluded that the oil would not be nutritionally disadvantageous to consumers compared to other marine oils.